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Award Number: DAMD17-00-1-0474

TITLE: Ph.D. Post-Doctoral Training Program in Breast Cancer Research

PRINCIPAL INVESTIGATOR: Dean P. Edwards, Ph.D.

CONTRACTING ORGANIZATION: University of Colorado Health Sciences Center
Aurora, Colorado 80045-6508

REPORT DATE: July 2001

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2001	Annual Summary (1 Jul 00 - 30 Jun 01)

4. TITLE AND SUBTITLE
Ph.D. Post-Doctoral Training Program in Breast Cancer Research

5. FUNDING NUMBERS
DAMD17-00-1-0474

6. AUTHOR(S)
Dean P. Edwards, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Colorado Health Sciences Center
Aurora, Colorado 80045-6508

E-Mail: dean.edwards@uchsc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

The training program is designed to graduate well-qualified and highly motivated scientists who will make a career in the breast cancer research field and who will have a strong potential for contributing new research approaches to the breast cancer problem. The students accepted into the program have already entered into different Ph.D. degree granting programs that each have their own guidelines, curriculums, and requirements. The curriculum of the Breast Cancer Training Program extends beyond that of the normal Ph.D. requirements to include didactic classroom teaching, journal clubs, seminars, workshops and mini-symposiums on relevant topics in breast cancer. Additionally, the program provides extensive one-on-one laboratory training in breast cancer research that is committed to the discovery of new fundamentals about the biology of breast cancer and its eventual treatment. The faculty who serve as research mentors have established records of successful training of Ph.D. and M.D./Ph.D. students.

14. Subject Terms (keywords previously assigned to proposal abstract or terms which apply to this award)	15. NUMBER OF PAGES
apoptosis, cell-cycle control, steroid hormone receptors, signal-transduction, oncogenes, steroid antagonists, breast cancer research	77
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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TRAINING AND KEY RESEARCH ACCOMPLISHMENTS

I Training

A) Recruitment and selection of students

The steering committee of the training program selected the first six students to participate in the training program and to be supported by the training grant. The students selected have already successfully completed the Core curriculum required for all Ph.D. students in the School of Graduate Studies and are in different stages of their thesis research on a breast cancer project with one of the training faculty. The students selected, the faculty mentors, and Ph.D. degree-granting programs are listed in the table below. It should be noted that four of the students are combined M.D./Ph.D. students. Dr. Ian McNiece was not listed as a training faculty on the original grant application. He has subsequently been added and is mentoring Steven Rosinski. Dr. McNiece's biosketch, other support, human subjects protocol, and safety plan are included in the Appendix.

Student	Type of Student	Faculty Mentor	Ph.D. Program
Lee, Richard	M.D./Ph.D.	Steve Anderson, Ph.D.	Pathology
Prescott, Jason	M.D./Ph.D.	Arthur Gutierrez-Hartmann, M.D./Ph.D.	Molecular Biology
Roemer, Sarah	Ph.D.	Dean Edwards, Ph.D.	Molecular Biology
Rosinski, Steven	M.D./Ph.D.	Ian McNiece, Ph.D.	Pharmacology
Spalding, Aaron	M.D./Ph.D.	Gary Johnson, Ph.D.	Pharmacology
Wu, Christine	Ph.D.	Margaret Neville, Ph.D./ Kathryn Howell, Ph.D.	Cellular & Structural Biology

B) Research seminars/mini-symposium/journal club/workshops

Students accepted into the training program have already entered into existing Ph.D. degree-granting programs in the University and must meet the requirements of the individual program. The curriculum of the breast cancer training program extends beyond that of the normal Ph.D. requirements to include a regular research seminar series, mini-symposium/journal club/workshops and a formal course on the biology and clinical aspects of breast cancer.

A breast cancer research seminar series for trainees and faculty was organized and initiated in the 2000-01 academic year. The seminar series interfaced with an existing seminar Program sponsored by the Hormone Related Malignancies Program of the UC Cancer Center. This seminar series is held weekly (Tuesdays 4-5 PM) and includes topics on all endocrine and hormone regulated cancers. Although all our students and faculty are encouraged to attend all of the seminars, there were 10 breast cancer research seminars throughout the year that the students and training faculty were required to attend. A schedule of the seminar series is included in the Appendix. The seminar speakers included a mix of training faculty, other faculty in the University, and outside invited speakers. Additionally, each of the six students gave a seminar on their research

progress at the end of the academic year in three special seminar sessions (see Appendix for seminar schedule).

An all day mini-symposium of outside invited speakers on various "hot topics" in breast cancer research was planned for each year of the training grant. Because this type of event needs considerable advance planning, it was not possible to organize this within the 2000-01 academic year. We are in the planning stages now for such a mini-symposium for February - March of the 2001-02 academic year.

A monthly journal club (2nd Friday of the month) to discuss current breast cancer topics in the original literature was initiated and held throughout the 2000-01 academic year. This was organized in conjunction with a related program project grant on developmental regulation of the normal mammary gland. This program project grant is headed by Dr. Margaret Neville, one of the training faculty of the breast cancer training grant. The journal club provides the unique opportunity for students and fellows to have lively discussions on normal mammary gland biology and how this information provides insights into mammary tumorigenesis (see Appendix for schedule).

One of our trainees, Sarah Roemer, attended the AACR-sponsored workshop in Keystone, Colorado (July 15-20th) on the " Pathobiology of Cancer." This is a valuable workshop designed for students to provide the fundamentals on cancer biology and pathology. We anticipate sending several more students to this workshop in future years.

A planned didactic course on the biology and clinical aspects of breast cancer, to be taught by the training faculty and other selected clinical faculty on campus, did not occur in the first year of the training grant. Again, because this type of activity needs considerable advance planning, it was not possible to organize this course within the 2000-01 academic year. The course as outlined in the grant application will take place in the Winter quarter 2002. The course will be open to all graduate students and will be required for the students in our training program.

II Research Accomplishments

The following are descriptions of the research accomplishments of each of the students over the last year.

Student Name: **Richard C.H. Lee**

Mentor/Principal Investigator: Steven M. Anderson, Ph.D.

Studies of prolactin and its cognate receptor (PRLR) utilizing knockout mice have demonstrated an important role for these proteins in mammary gland development and potentially in tumorigenesis. Over the past year, Richard Lee has been studying the role of the PRLR in mammary gland development by using transgenic mice that overexpress a constitutively active deletion mutant of PRLR (Δ178Flag). The mammary glands of transgenic mice show precocious pubertal development in virgin stages (5 and 10 weeks) as well as early

pregnancy (day 6), when compared to the mammary glands of control mice. An examination of the 5-week old virgin mammary glands using whole mounts showed that the numbers of terminal end buds, terminal ducts, and alveolar buds increased in transgenic mice. Transgenic mammary glands at 10 weeks also exhibited more early alveolar structures, increased ductal branching, and increased epithelial cell content. In early pregnancy, transgenic mammary glands exhibited increased ductal branching as well as a higher degree of alveolar development. Injection of estrogen, progesterone or estrogen plus progesterone indicated that transgene expression was responsible for these developmental changes and not the hormonal status of the mouse. In addition, involution was delayed in the mammary glands of transgenic mice. These mammary glands exhibited more lobuloalveolar structures and an increase in the ratio of mammary epithelial cells to adipocytes at involution day 4 and 6. By day 8, both control and transgenic mammary glands looked similar. These data indicate that constitutively active PRLR induces precocious development in virgin and pregnant mammary glands as well as delays in involution, suggesting that PRLR plays an important role in mammary gland proliferation and development. These data also suggest that mutation of the PRLR such that it becomes constitutively activated could contribute to the development of breast cancer. Transgenic mice are being followed for longer times and are being crossed with other mice to determine whether the mutant PRLR plays a role in mammary tumorigenesis. Although deletion mutations in the PRLR have been reported in human breast cancer cell lines and tissue samples, the analysis of these mutants has not included a complete biological/biochemical characterization that would determine whether these molecules contribute to breast tumorigenesis.

Student Name: **Jason D. Prescott**

Mentor/Principal Investigator: Arthur Gutierrez-Hartmann, M.D./Ph.D.

ESX is a novel member of the proto-oncogenic Ets transcription factor family that appears to mediate normal involution in the postpartum breast. Furthermore, ESX over-expression is detected in 40% of human breast ductal carcinomas in situ. Stable over-expression of ESX also enhances survival of nontransformed human breast cells in culture, while inhibition of ESX activity decreases breast cancer cell viability. ESX function depends on concurrent binding of ESX protein both to a specific regulatory element in a target gene and to an adjacent, as yet unidentified, DNA-binding protein cofactor. The resulting complex then modulates transcription of the target gene. *In vitro*, ESX specifically binds and trans-activates several breast cancer-related gene promoters, including those of the human HER-2 (c-erb-b2) and TGFb RII genes.

Over-expression of the normal HER-2 transmembrane receptor plays a central causal role in the development of some human breast cancers. Further, a subset of these cancers over-express this protein in the absence of mutation in the HER-2 gene. This suggests that dysfunction in the regulation of HER-2 is oncogenic. Given that ESX stimulates expression of HER-2 and that ESX is itself over-expressed in some human breast cancers, Jason's research seeks to confirm the hypothesis that inappropriate ESX expression results in excessive HER-2 expression and thus plays a pivotal, causal role in the development of some human breast cancers. During the last year, Jason's research has demonstrated specific, physiologic binding between endogenous ESX protein and the endogenous HER-2 promoter in cultured human breast cells. This was accomplished using an anti-ESX antibody in the chromatin immunoprecipitation

(ChIP) assay. He has also shown that expression of exogenous ESX protein in nontransformed human breast cancer cell lines stimulates induction of HER-2 protein expression (average of 2.2 fold.) These data represent significant steps in demonstrating a direct stimulatory role for ESX protein on HER-2 gene expression in human breast cells and thus suggest a causal role for ESX in the development of HER-2 positive breast cancer.

Student Name: **Sarah C. Roemer**

Mentor/Principal Investigator: Dean Edwards, Ph.D.

The steroid receptor progesterone receptor (PR) has been implicated in the development and progression of both breast and uterine cancer. Previous work from the Edwards lab has shown that the chromatin associated high mobility group proteins 1 and 2 (HMG-1/-2) enhance the affinity of progesterone receptor (PR) for progesterone response elements (PRE) *in vitro*, and the transcriptional activity of PR *in vivo*. HMG-1/-2 is also an important coregulatory protein for estrogen receptor (ER) and other steroid hormone receptors. Additionally, the level of HMG-1/-2 expression in breast cancer cells can significantly alter the pharmacology of the anti-estrogen tamoxifen, thus implicationg HMG-1/-2 as a factor involved in determining tumor response and tamoxifen therapy. The mechanism of enhancement of PR-DNA (or other steroid receptors) binding is not well defined. It is thought the PR-DNA complex recruits HMG-1/-2 to provide additional protein-protein and/or protein-DNA contacts. Structure analysis will be required to precisely determine how HMG interacts with the PR-DNA complex to affect the affinity of PR for DNA. Over the past year Sarah Roemer has worked on obtaining a purified HMG-PR-DNA ternary complex for crystallography. To obtain a suitable complex first required determination of the minimal domains of PR and HMG required for interaction. HMG-1/-2 are composed of two homologous, but not identical, DNA binding domains termed HMG box A and B, a basic region immediately C-terminal to each box, and an acidic C-terminal tail. Domains derived from HMG-1 and the DBD of PR were expressed and purified to near homogeneity for use in functional analysis. Both HMG boxes with or without the basic regions were able to enhance binding of the PR-DBD to PREs in electrophoretic mobility shift assays. HMG boxes containing the basic regions were found to be capable of binding to PREs more tightly than the boxes alone and full length HMG 1/-2. This result suggests that the basic regions are involved in DNA binding and that the acid tail interferes with DNA binding in the full length HMG protein. An HMG DNA binding deficient box B construct, containing basic region, is capable of enhancing DBD binding as efficiently as wild type box B, suggesting that HMG binding to DNA is not necessary to enhance PR-DNA binding. In GST pull down assays, an HMG-1 construct containing both box A and B and full-length HMG-1 showed direct protein interaction with full length PR and the PR-DBD. The interaction between HMG-1 and PR-DBD was inhibited by the presence of specific DNA. However, the presence of DNA does not prevent the interaction between HMG-1 and full length PR indicating that additional contacts made outside of the DBD of PR are required for formation of a stable ternary complex. This information will be used in the upcoming year to design the appropriate PR constructs to form a ternary PR/DNA/HMG complex so that crystallography trials can begin.

Student name: **Steven Rosinski**

Mentor/Principle investigator: Ian McNiece, Ph.D.

Five-year relapse free survival for metastatic breast cancer following high-dose chemotherapy and hematopoietic stem cell rescue is 45%. A possible direction to improve relapse free survival is the development of strategies to utilize the immune system to eliminate the residual tumor after transplant. Immunodeficiency due to insufficient T cell recovery remains a significant barrier to the development of effective immunotherapy in patients that have undergone cytoreductive therapy. Mouse experiments suggest that thymus independent mechanisms maybe responsible for a component of T cell reconstitution in adults following cytoreductive therapy. Over the past year, Steve Rosinski has tested this hypothesis by enumerating T cell and three distinct blood dendritic cell levels from patients with metastatic breast cancer before and sequential times post autologous transplant. Preliminary analysis in the majority of patients shows a return of CD8+ T cell levels within 30 days, however, CD4+ T cell levels have not returned for a year or longer following transplant an observation consistent with previous reports. Additionally, naïve CD8+ T cell levels but not CD4+ T cells return after transplant. Also, a V-beta analysis on the reconstituted CD4 and CD8 T cell populations has shown a skewing of the repertoire further analysis is ongoing. Patients that have deficient CD4+ T cell levels have CMRF44+ DC levels that are ten fold lower then patients who have reconstituted their CD4+ T cell levels. This suggests a role for CMRF44+ DC in thymus independent T cell maintenance. The ability of blood dendritic cells to reconstitute CD4+ T cells has major implications for the development of immunotherapuetic strategies directed at preventing infectious complications and improving relapse free survival.

Student Name: **Aaron C. Spalding**

Mentor/Principal Investigator: Gary L. Johnson, Ph.D.

TNF-related apoptosis inducing ligand, TRAIL, is a recently cloned cytokine that has been shown to induce apoptosis in a synergistic fashion with chemotherapeutic agents on several cancer cell lines. Xenografts of several carcinoma cell lines demonstrate TRAIL and chemotherapy to cause complete regression of established tumors. Over the past year, Aaron Spalding has shown that the DNA damaging agents etoposide and doxorubicin, induce the upregulation of TRAIL in several breast carcinoma lines as well as non-transformed non-immortalized human mammary epithelium. Analysis of human adenocarcinomas demonstrates TRAIL mRNA expression is significantly decreased in tumor relative to autologous non-tumor tissue. Furthermore, genotoxin-induced apoptosis of human breast cancer cells correlates with genotoxin induced TRAIL surface expression. Using a dominant negative inhibitor of NFκB, Aaron has shown in ZR-75-1 breast cancer cells that expression of TRAIL and its death receptor, DR5, is regulated by NFκB. Inhibition of NFκB results in aggressive growth and chemotherapy resistance of human carcinomas xenografted onto nude mice. Aaron also performed gene profiling of human cancer cells to demonstrate that NFκB regulates the expression of both pro- and anti-apoptotic proteins including inhibitors of apoptosis (IAPs) in addition to DR5 and TRAIL. It is the predominance of the opposing NF-κB-dependent signals that dictates the cell's

decision to survive or die. Loss of TRAIL expression in human cancer provides a means by which tumors can avert programmed cell death.

Aaron's other work has focused on the role of DcR1, a GPI-linked TRAIL receptor, in resistance of breast cancer cells to DNA damaging agents. DcR1 has been proposed to confer TRAIL resistance by serving as a membrane bound TRAIL trap. His experiments demonstrate DcR1 expression inhibits caspase 8 and BID cleavage as well as JNK phosphorylation induced by etoposide and TRAIL. DcR1, however, also induces potent survival signals. Restoring DcR1 in breast carcinoma cells to physiologic levels results in Akt activation in a src family kinase dependent manner. Additionally, DcR1 expression confers resistance to mitochondria insult and apoptosis induced by currently utilized chemotherapeutic agents as well as to Antimycin A, a BH3 domain homologue that targets mitochondria directly. These data indicate that in addition to inhibiting DR4 and DR5 signaling by binding TRAIL, DcR1 also influences cell survival by preserving mitochondrial integrity. DcR1 is a regulator of the apoptosis module whose expression enhances cell survival and confers resistance to chemotherapy. This work clearly demonstrates the importance of TRAIL and its negative regulator, DcR1 in breast cancer response to currently used chemotherapeutics as well as potential novel drugs which target mitochondria directly. Further characterization of the TRAIL system in cancer is needed to determine the clinical influence of this cytokine on both neoplastic transformation as well as resistance of carcinoma to current treatments.

Student Name: **Christine C. Wu**

Mentor/Principle Investigator: Kathryn E. Howell, Ph.D./ Margaret Neville, Ph.D.

A major challenge in the study of cancer cells is to identify the cast of signaling proteins and to compare and contrast this list with the protein repertoire of a normal cell. During this past year, Christine Wu's project has been to determine if proteomics could be used to determine the differences in protein expression and post-translational modifications between two developmental states of the mammary epithelial cell population. Epithelial cell populations were first isolated and enriched from late pregnant and mid-lactation rat mammary tissue. The cells were fractionated, and subcellular fractions were characterized biochemically and morphologically to evalulate enrichment. Comparative proteomics was then applied to study the molecular protein changes of two normal cellular processes (initiation of copious protein production/secretion and the initiation of lipid secretion) during lactogenesis II (the transition between pregnancy and lactation). These comparisons were conducted using two-dimensional gel electrophoresis to resolve and visualize the samples and tandem mass spectrometry to identify the resolved proteins on the gel. Key players involved in the expansion of the Golgi complex and the initiation of milk lipid secretion were identified (Wu *et al*, 2000a; Wu *et al,* 2000c). Having evaluated proteomic technology in normal rat mammary tissue, the lab is now in the process of shifting gears to compare and contrast normal and diseased states of fractionated human breast tissue. The use of proteomics to complement the vast amounts of microarray data already published to date will greatly expand our knowledge because, not only will the proteins be identified by subcellular localization, but the post-translational modifications (especially phosphorylation) can be determined. The establishment of protein databases of the diseased and normal state by organelles would greatly contribute to the understanding of the multiple signaling pathways involved. The comparisons of these protein profiles provide novel insight

into key functional players and the molecular mechanisms of the diseased state as well as provide a method to identify unique protein markers.

OUTCOMES RESULTING FROM TRAINING GRANT AWARD

I Presentations/Abstracts at National Meetings

Student: **Richard Lee**

MM Richert, **RC Lee**, SM Anderson. Expression of Constitutively Active Prolactin Receptor in Mouse Mammary Glands Induces Precocious Pubertal Development and Delays Involution. The Endocrine Society's 83rd Annual Meeting, Abstract #ORI5-1, p. 87, 2001.

Lee, R., Richert, M., Schaack, J., and Anderson, S. Activation of Signaling Pathways by Constitutively Active Prolactin Receptor using Adenoviral Transduction. Prolactin-Gordon Research Conference, 2000.

Lee, R., Richert, M., Schaack, J., and Anderson, S. Activation of Signaling Pathways by Constitutively Active Prolactin Receptor using Adenoviral Transduction in Mouse Mammary Epithelial Cell Lines. The 15th Annual National MD/PhD Conference, Aspen, CO, 2000.

Student: **Jason Prescott**

JD Prescott, A.Gutierrez-Hartmann. Analysis of ESX Function in the Development of Human Breast Cancer. The 83rd Annual Endocrine Society Meeting, Abstract #P3-672, p. 591, June 2001, Denver, Colorado.

Student: **Sarah Roemer**

Roemer, S.C., Adelman, J., Churchill,M., Edwards,D.P. Mechanism of HMG-1/-2 Enhancement of Progesterone Receptor Binding to Target DNA. Annual Endocrine Society Meetings, Denver, Colorado. Abstract # P1-69, p. 164, 2001.

Roemer, S.C., Adelman, J., Churchill,M., Edwards,D.P. Mechanism of HMG-1/-2 Enhancement of Progesterone Receptor Binding to Target DNA. AACR Pathobiology of Cancer Workshop, Keystone, Colorado, 2001.

Student: **Steven Rosinski**

S. Rosinski, P. Kerzic, R. Quinones, E.J. Shpall, M. Malatchi, P. Russel, J. McDermott, D. Hart I. McNiece. Allogeneic cord blood recipients have delayed immune recovery associated with a deficiency in dendritic cells. Experimental Hematology Annual Meeting, 2000

S. Rosinski, P. Kerzic, R. Quinones, E.J. Shpall, M. Malatchi, P. Russel, J. McDermott, D. Hart I. McNiece. Allogeneic cord blood recipients have delayed immune recovery associated with a

deficiency in dendritic cells. International Society of Hematopoietic Therapy and Graft Engineering Meeting, 2000

Student: **Christine Wu**

Howell KE and **Wu CC**. Proteomics and High Resolution 3D Reconstruction of the Golgi Complex in the Mammary Epithelial Cell. Mammary Gland Gordon Conference. Bristol, Rhode Island, June, 2001.

II Publications

Student: **Aaron Spalding**

Spalding, AC., Jotte, R., Chamberlain, W., and Johnson, G.L. TRAIL Decoy R1 Downregulates the Apoptotic Module Coupled with Akt Activation to Attenuate Tumor Cell Apoptosis. Science, submitted.

Spalding, A.C., Jotte, R.M., Sugita, M., Gao, B., Geraci, M. W., Franklin, W. and Johnson, G.L. (2001) NFkB Regulates TRAIL and IAPs To Determine The Response of Breast Cancer Cells to Genotoxins. Nature Medicine, in review.

Student: **Christine Wu**

Wu CC, Howell KE, Neville MC, Hays LG, Yates JR III, McManaman JL. Proteomics reveal a link between the endoplasmic reticulum and lipid secretory mechanisms in mammary epithelial cells. Electrophoresis 21: 3470-3482, 2000.

Wu CC, Taylor RS, Lane DR, Ladinsky MS, Weisz JA, Howell KE. GMx33: a novel family of trans-Golgi proteins identified by proteomics. Traffic 1: 963-975, 2000.

Wu CC, Yates JR III, Neville MC, Howell KE. Proteomic analysis of two functional states of the Golgi complex in mammary epithelial cells. Traffic 1: 769-782, 2000.

III Degrees Awarded

Chris Wu and Richard Lee successfully defended their thesis research and will be graduating in August, 2001. Chris Wu will be taking a postdoctoral fellowship with Dr. John Yates, III in the Department of Cell Biology at the Scripps Research Institute. Richard Lee will return to medical school at UCHSC (3^{rd} and 4^{th} year) to complete his clinical training.

IV Data Bases

Chris Wu and her mentors are creating genomic and proteomic data bases for organelle proteins in normal mammary gland and in breast cancer and for organelles at different stages of normal mammary gland development.

Biographical Sketches

Provide the following information for the key personnel listed on the budget page for the initial budget period.

NAME	POSITION TITLE
McNiece, Ian K.	Professor

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include post-doctoral training.)

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
Melbourne University, Melbourne, Australia	B.S.	1979	Biochemistry
Melbourne University, Melbourne, Australia	Ph.D.	1986	Hematology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past 3 years and to representative earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. PAGE LIMITATIONS APPLY. DO NOT EXCEED 3 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

Employment/Experience

1986 (Jan.-July)	Postdoctoral Fellow - Cancer Institute, Melbourne, Australia.
1986-1988	Postdoctoral Fellow – University of Virginia, Charlottesville, Virginia
1988-1994	Research Scientist – Amgen, Inc., Thousand Oaks, California
1994-1997	Laboratory Head, Dept. of Developmental Hematology, Amgen, Inc., Thousand Oaks, California
1997-Present	Director of Research, Bone Marrow Transplant Program and Professor of Medicine, Universeity of Colorado Health Sciences Center, Denver, CO
1998-Present	Joint appointment, Professor, Dept. of Pharmacology, Dept. of Immunology, University of Colorado Health Sciences Center, Denver, CO

Professional Activities

1993-Present	Editorial Board, Stem Cells
1998-Present	Associate Editor, Ezperimental Hematology
1997-Present	ISHAGE Ex Vivo Expansion Committee
1999	Scientific Advisory Board – 28th Annual Meeting of ISEH
1988-Present	Laboratory Inspector – FAHCT Transplant Program Accreditation
2000	Program Advisory Board – 10th Int. Symposium on ABMT
2000-Present	Membership Committee of ISEH
	Ad hoc member, Hematology I Study Section, NIH

Selected Publications (Total of 78 peer reviewed articles and 42 Chapter and review articles)

McNiece IK, Bradley TR, Kriegler AB, Hodgson GS (1986). Subpopulations of mouse bone marrow high proliferative potential colony forming cells (HPP-CFC). Exp.Hematol. 14(9): 856-860.

Bertoncello I, Bartelmez SH, Bradley TR, Stanley ER, Harris RA, Sandrin MS, **McNiece IK**, Kriegler AB, Hunter SD, Hodgson GS (1986). Isolation and analysis of primitive hemopoietic progenitor cells on the basis of differential expression of Qa-m7 antigen. J.Immunol. 136(9): 3219-3224.

McNiece IK, Williams NT, Johnson GR, Kriegler AB, Bradley TR, Hodgson GS (1987). Generation of murine hematopoietic precursor cells from macrophage high-proliferative-potential colony forming cells. Exp. Hematol. 15(10):972-977.

RESEARCH AND PROFESSIONAL EXPERIENCE (CONTINUED). PAGE LIMITATIONS APPLY.
DO NOT EXCEED 3 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

McNiece IK, Stewart FM, Deacon DM, Quesenberry PJ (1988). Synergistic interactions between hemopoietic factors as detected by in vitro mouse bone marrow colony formation. Exp.Hematol.16:383.

McNiece IK, Stewart FM, Deacon DM, Temeles D.S., Zsebo KM, Clark S, Quesenberry PJ (1989). Detection of a human CFC with a high proliferative potential. Blood 74(2):609-612.

Keller JR, **McNiece IK**, Sill KT, Ellingsworth LR, Quesenberry PJ, Sing GK, Ruscetti FW (1990). Transforming growth factor B regulates primitive murine hematopoietic cell proliferation. Blood 75(3):596-602.

McNiece IK, Langley KE, Zsebo KM (1991). The role of recombinant stem cell factor in early B cell development: synergistic interaction with IL-7. J. Immunol. 146(11):3785-3790.

Williams N, Bertoncello I, Kavnoudias H, Zsebo K, **McNiece I** (1992). Recombinant rat stem cell factor stimulates the amplification and differentiation of fractionated mouse stem cell populations. Blood 79(1):58-64.

Zsebo KM, Smith KA, Hartley CA, Greenblatt M, Cooke K, Rich W, **McNiece IK** (1992). Radioprotection of mice by recombinant rat stem cell factor. PNAS 89:9464-9468.

Briddell RA, Hartley CA, Smith KA, **McNiece IK** (1993). Recombinant rat stem cell factor synergizes with recombinant human granulocyte-colony stimulating factor in vivo in mice to mobilize peripheral blood progenitor cells which have enhanced repopulating ability. Blood 82(6):1720-1723.

Samal B, Sun Y, Stearns G, Xie C, Suggs S, **McNiece I** (1994). Cloning and characterization of the complementary DNA encoding a novel human pre-B cell colony enhancing factor. Mol.Cell.Biol. 14:1431-7.

Andrews RG, Briddell RA, Knitter GH, Opie T, Bronsden M, Myerson D, Appelbaum FR, **McNiece IK** (1994). In vivo synergy between recombinant human stem cell factor and recombinant human granulocyte colony-stimulating factor in baboons: enhanced circulation of progenitor cells. Blood 84(3):800-810.

Yan X-Q, Briddell R, Hartley C, Stoney G, Samal B, **McNiece I** (1994). Mobilization of long-term hematopoietic reconstituting cells in mice by the combination of SCF plus G-CSF. Blood 84(3):795-799.

Andrews RG, Briddell RA, Knitter GH, Rowley SD, Appelbaum FR, **McNiece IK** (1995). Rapid engraftment by peripheral blood progenitor cells mobilized by recombinant human stem cell factor and recombinant human granulocyte colony-stimulating factor in nonhuman primates. Blood 85(1):15-20.

Yan X-Q, Hartley C, McElroy P, Chang A, McCrea C, **McNiece I** (1995). PBPC mobilized by rhG-CSF plus rrSCF contain long-term engrafting cells capable of cellular proliferation for over 2 years as demonstrated by serial transplantation in mice. Blood 85(9):2303-2307.

Yan X-Q, Lacey D, Fletcher F, Hartley C, McElroy T, Sun Y, Xia M, Mu S, Saris C, Hill D, Hawley RG, **McNiece I** (1995). Chronic exposure to retroviral vector encoded MGDF (mpl-ligand) induces lineage-specific growth and differentiation of megakaryocytes in mice. Blood 86(11):4025-4033.

Molineux G, Hartley C, McElroy P, McCrea C, **McNiece IK** (1996). Megakaryocyte growth and development factor accelerates platelet recovery in peripheral blood progenitor cell transplant recipients. Blood 88(1):366-376.

Yan XQ, Lacey D, Hill D, Chen Y, Fletcher F, Hawley R, **McNiece IK** (1996). A model of myelofibrosis and osteosclerosis in mice over-expressing MGDF (mpl-ligand): reversal of disease by bone marrow transplantation. Blood 88(2):402-409.

Molineux G, Hartley C, McElroy P, McCrea C, **McNiece IK** (1996). Megakaryocyte growth and development factor stimulates enhanced platelet recovery in mice after bone marrow transplantation. Blood 88(4):1509-1514.

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GG Principal Investigator/Program Director *(Last, first, middle):* McNiece, Ian, K,

Other Support:

ACTIVE

1. RO1#CA615082 (Shpall) Co-Investigator 7/1/97 - 6/30/02 10%
   NIH $125,000/year
   Title: Hematopoietic Progenitor Cells
   The major goal of this project is to determine the clinical potential of ex vivo expanded peripheral blood progenitor cells in breast cancer patients.

2. RO1# (Hogan) 7/1/99-6/30/02 5%
   Co-Investigator
   NIH $200,000/year
   Characterization of human hematopoietic precursor cells.

3. NM DP (McNiece) PI 2/1/00 - 9/30/01 20%
   Natonal Marrow Donor Program $208,000 over 2 years
   Title: The role of Cord Blood T cells in Allogeneic Transplant
   The goal of this project is to study immune reconstitution in recipients of ex vivo expanded CB and to evaluate the potential of CB T cells to induce an anti tumor effect.

4. Ontogeny (McNiece & Hogan) 3/1/00 - 9/30/01 0%
   PI $74,825
   Title: Stem cell isolation
   The goal of this project is to isolate purified hematopoietic stem cell populations for screening of novel genes.

5. Ontogeny (McNiece) 1/1/01 - 12/31/01 0%
   PI $9,500
   Title: Overexpression of hedghog
   The goal of this project is to overexpress the sonic hedgehog gene for studies on the biologic function of the gene in vivo.

6. RO1#CA8887801(McNiece) PI 1/1/01 - 12/31/05 30%
   NIH $260,814/year
   Title: Ex vivo expanded cord blood cells
   The goal of this project is to optimize culture conditions for the ex vivo expansion of CB cells and to evaluate these conditions in clinical trials. The studies will also comprehensively evaluate immune reconstitution and the potential of cord lymphocytes in induction of an anti tumor effect.

7. Immunex Corp (McNiece) 1/1/01 – 12/31/04 20%
   PI $150,000/year
   Title: Over expression of novel genes in vivo
   The goal of this project is to overexpress novel genes identified from genomic screening in hematopoietic stem cells in vivo to identify potential biology of gene products.

Pending:

1. STTR (McNiece) PI 7/1/01-6/30/03 10%
   NIH $100,000/year

Title: Expansion of CB cells on MSC

The goal of this project is to determine the optimal conditions for ex vivo expansion of cord blood mononuclear cells on mesenchymal stem cells.

Trainee	Steven Rosinski
Pre/Post	Pre
Training Period	10/1/98 to present
Prior Academic Degree	BSc/University of Arizona, 1996
Title of Research Project	Immune recovery following hematopoietic stem cell transplantation
Current Position	Graduate Student (DOD Fellowship grant)

Trainee	Rohaizah James
Pre/Post	Post
Training Period	4/1/01 to present
Prior Academic Degree	PhD/University of Wisconsin, Madison, WI 1994
Title of Research Project	Gene marking of ex vivo expanded cord blood
Current Position	NIH RO1

Trainee	Sherilyn Gross
Pre/Post	Post
Training Period	1998 to 2000
Prior Academic Degree	PhD/ University of Colorado HSC/1998
Title of Research Project	Cord blood T cells as a source of DLI
Current Position	Post doctoral fellow, UCHSC

Trainee	Zonghan Dai
Pre/Post	Post
Training Period	1998 to 1999
Prior Academic Degree	PhD/Univ of N Carolina, Chapel Hill , NC/1992
Title of Research Project	Abl-Abi signalling
Current Position	Assistant Professor UCHSC

TITLE: **Transplantation of Breast Cancer Patients with Growth Factor Mobilized Peripheral Blood Progenitors Which are CD34-Selected and Expanded Ex Vivo Prior to Infusion (IRB #98-040)**

Principal Investigators: Elizabeth J. Shpall, MD
Ian McNiece, PhD
Bone Marrow Transplant Program
University of Colorado Health Sciences Center
4200 E. 9th Avenue, Box B190
Denver, CO 80262
(303) 372-9000 - phone
(303) 372-9003 - fax
elizabeth.shpall@UCHSC.edu

Co-Investigators: Scott I. Bearman, MD
Pablo J. Cagnoni, MD
Wilbur Franklin, MD
Roy B. Jones, PhD, MD
Peter McSweeney, MD
Yago Nieto, MD

Statistical Consultant: James Murphy, PhD

STUDY APPROVAL	**DATE**
COMIRB	
Initial Approval	04/17/98
Amendment #1	06/15/98
Amendment #2	01/15/99
Resubmission to COMIRB	03/27/00
Continuing Review	08/07/00
Continuing Review (7/13/01)	

TABLE OF CONTENTS

PROTOCOL SYNOPSIS

Estimated Duration of Study:	Accrual Follow-Up	2 years 3 years
Subjects of Amendment:	Patients	
Number of Patients:	45-60	
Sex:	Female	
Age Range:	18-65	
Uses ionizing radiation?	No	
Amendment involves use of Durable Power of Attorney?	No	
Off-site project?	No	
Multi-Institutional Project?	No	

I. SPECIFIC AIMS

To comprehensively evaluate the use of autologous peripheral blood progenitor cells (PBPCs), which have been CD34-selected and expanded ex vivo prior to infusion, as hematopoietic support for patients with breast cancer.

A. To determine the rate and duration of hematopoietic reconstitution produced by growth factor-mobilized PBPCs which are immunomagnetically CD34 selected and then expanded ex vivo prior to transplantation

B. To evaluate the amount of breast cancer in the PBPC fractions before and after each CD34-selection and ex vivo expansion procedure, using the following assays:

1. Routine hematoxylin and eosin (H&E) staining
2. Immunocytochemistry
3. Polymerase chain reaction (PCR) for cytokeratin-19 (K19) and muc-1

Table 1. Breast Cancer Clonogenic Liquid Culture Assay

N = 32	Culture Positive N = 20	Culture Negative N = 12
ICC Positive	11	1
ICC Negative	9	11

II. BACKGROUND AND RATIONALE

Rationale for Purging. A potential limitation of autologous hematopoietic support is the presence of clonogenic tumor in the hematopoietic progenitor cell fractions, which could produce relapse in the patients posttransplant. This is of particular concern for patients with diseases such as breast cancer, which commonly involve the marrow.[1-3] While no definitive evidence exists, studies such as those of Gribben et al.,[4] Sharp et al.,[5] and Brenner[6] suggest that infusion of tumor in an autograft may contribute to relapse of disease. Over the past few years, PBPCs have been employed as a substitute for marrow in patients with known marrow metastases because of the belief that peripheral blood contained fewer tumor cells than the corresponding marrow. Although this may still be the case, the recent development of sensitive detection techniques have revealed that contamination of peripheral blood with breast cancer is common and much more prevalent than routine histology would suggest.[5,7-9]

Detection of Minimal Residual Breast Cancer in Marrow and PBPC Autografts. Currently, immunocytochemical staining is most the commonly used technique for the identification of breast cancer cells in bone marrow, peripheral blood, and/or PBPC fractions.[2,3,10-15] The assay has the advantage of specific immunocytochemical markers, in addition to morphology, for the identification of breast cancer cells in hematopoietic specimens. We developed a quantitative immunocytochemical assay, with a sensitivity of

one breast cancer cell in 3 x 10^6 marrow or PBPCs, which we have used over the past several years to detect breast cancer in autografts before and after graft manipulation.[10] More recently, we have been evaluating reverse transcriptase polymerase chain reaction (RT-PCR) assays that identify the cytokeratin and muc-1 genes expressed by breast cancer cells with sensitivities which are similar to our immunocytochemical assay. The inability to assess the clonogenic potential of breast cancer cells with immunocytochemical or molecular assays makes the interpretation of their clinical relevance difficult. After a decade of unsuccessful attempts, we have recently developed an ex vivo culture method to assess the clonogenic potential of breast cancer cells found in marrow.[16] Out of eight different culture conditions, maximal breast cancer cell growth was documented when $1.25x10^5$ marrow mononuclear cells (MNCs)/cm^2 were cultured for 14 days in collagen-coated petri dishes containing RPMI 1640 and 10% FBS. We cultured aliquots of marrow from 32 consecutive breast cancer patients using those conditions, and found that 20 of them had breast cancer cells detected by day 14. As shown in Table 1, nine of the 20 culture-positive patients had no detectable breast cancer by any other method, including our routine H&E, immunocytochemical (ICC), or molecular assays. These results suggest that we are probably grossly underestimating the level of breast cancer cell contamination in hematopoietic cell autografts using current technology. Ex vivo clonogenic cultures may enhance the detection of breast cancer cells.

Purging/Purification Studies. There are many different techniques currently employed to purge hematopoietic cell grafts. Negative purging methods where hematopoietic cell grafts are treated with immunologic[17] and/or pharmacologic[18] agents to remove malignant cells, have been shown to play a role in reducing relapse in patients with hematologic malignancies.[4,19,20] These methods are also associated with delayed engraftment. Positive-selection methods employ the CD34 antigen expressed on both the pluripotent and committed hematopoietic progenitors, but not on the majority of tumor cell types, including breast cancer.[21] Several different positive methods are being investigated; the majority of which involve separation of the target $CD34^+$ cells on relatively large macroscopic immunospecific surfaces, including plastic plates,[22] columns,[18] or magnetic beads.[23]

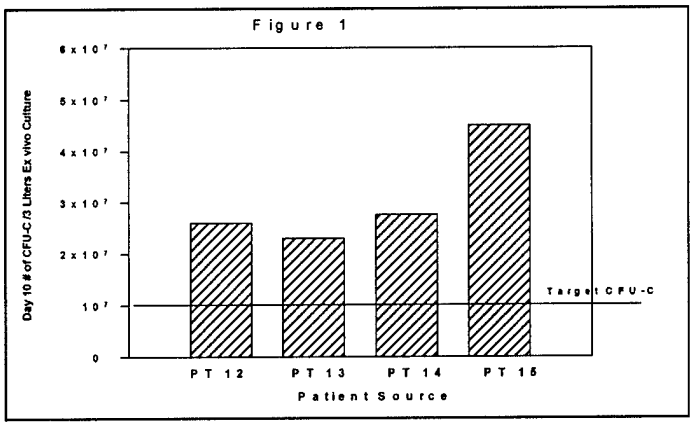
Over the past six years, our laboratory has evaluated several different clinical-scale breast cancer purging methods, including the CellPro immunoadsorption columns,[24,25] both the AmCell[26] and Baxter immunomagnetic columns,[27] and the COBE density gradient system.[27] Preliminary data suggests that combining two different purging procedures results in more effective eradication of breast cancer when compared to one purging modality, but substantial $CD34^+$ cell losses also occur. Strategies to increase the number of normal hematopoietic cells in a purged autograft are needed. If such strategies could also provide additional anti-tumor efficacy, the clinical outcome of breast cancer patients receiving autologous transplants could potentially be improved. This study will focus on the ex vivo expansion of PBPCs in an attempt to eradicate residual breast cancer from purged autografts, while increasing the number of normal hematopoietic progenitors, to compensate for losses resulting from the preceding purging/purification procedure(s).

Rationale for the Ex Vivo Expansion of PBPCs as a Tumor Purging Strategy. Long-term culture of marrow from patients with leukemia has been shown to differentially support the growth and expansion of normal progenitors, while leukemic cells did not survive.[28-34] These data allow for the possibility that the malignant clone could be eradicated by culturing CML patients= marrow. Although long-term culture of CML marrow in early clinical studies did not result in complete elimination of disease in most patients,[35] it is likely that refinement of these early culture techniques will be necessary for complete eradication of the malignant clone. Verfaillie et al.,[30] demonstrated that when marrow cells from CML patients expressing $CD34^+$ HLA-DR- phenotype were cultured ex vivo, they did not exhibit the Philadelphia chromosome or the corresponding bcr/abl mRNA. Studies employing acute myeloid leukemia (AML) cell lines show a preferential malignant cell differentiation and growth inhibition relative to normal progenitors, when cultured in the presence of vitamin D.[32] Widmer et al., CD34-selected and then cultured ex vivo the marrow specimens from nine non-Hodgkin=s lymphoma patients whose tumors expressed bcl-2 [the t(14;18)] translocation] in polymerase chain reaction assays.[33] Seven of the nine specimens remained $bcl\text{-}2^+$ after CD34-selection. Following a 14-day incubation in liquid cultures containing growth factors, six of the seven samples became bcl-2 negative.

Primary breast cancer cells, especially those obtained from bone marrow or blood, are difficult to keep alive ex vivo, particularly in culture conditions that support the growth of hematopoietic progenitors.[34] Preliminary data from our group[36] and others[37] suggests that breast cancer identified in marrow or PBPC fractions is eliminated or reduced, following ex vivo culture. Other potential clinical applications of ex vivo expansion include the generation of sufficient progenitors to supplement an autograft or allograft, and perhaps ablate or markedly shorten the period of myelosuppression, particularly for heavily pretreated patients; generating sufficient stem cells from one marrow aspirate or pheresis procedure, thus reducing the need for large-scale harvesting of marrow or multiple leukaphereses; generating large volumes of immunologically active cells, such as dendritic cells, with antitumor activity to be used in immunotherapeutic regimens; and increasing the pool of stem cells which could be targets for the delivery of gene therapy.

Preclinical Expansion Studies. Haylock et al., reported that $CD34^+$ PBPCs cultured in media with interleukin-1β (IL1β), IL3, IL6, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), and stem cell factor (SCF) expanded the myeloid progenitors, measured as colony forming units-granulocyte macrophage (CFU-GM), a median of 66-fold over 14 days.[38] Many investigators have reported similar results with a variety of growth factor regimens which included erythropoietin (Epo) and/or Flt-3 ligand, in addition to those mentioned above.[39-41] For adequate expansion, these static culture systems all require multiple growth factors and progenitors which are enriched for $CD34^+$ cells, prior to culture.

We conducted a series of preclinical studies and determined that $CD34^+$ PBPCs (isolated with the CellPro device), cultured in teflon bags with defined media and SCF, IL-3, IL-6, and G-CSF (Amgen) produced the highest number of CFU-GM with the growth factors that were available to us at the time. PBPCs were then obtained from four breast cancer patients who donated an extra PBPC product (from a single leukapheresis procedure) for research. The $CD34^+$ cells were isolated and placed in culture under the optimal conditions defined above. All four patients had breast cancer cells immunocytochemically detected in their $CD34^+$ PBPC fractions. After 10 days in culture, the number of CFC generated in all four clinical-scale cultures was 2.3 - 4.5 times higher than the predetermined target of 1×10^7. The predetermined target was chosen because all patients in the CellPro trial who received $\geq 1 \times 10^7$ CFCs in their CD34-selected PBPC grafts engrafted promptly. Three of the four patients had no detectable breast cancer in their postculture fractions, while the fourth patient had detectable tumor at a reduced level (a 2-log depletion following culture was documented).



These data suggest that ex vivo expansion may be an effective clinical purging strategy. The clinical PBPC expansion studies proposed in the current study will comprehensively address this issue using $CD34^+$ PBPCs cultured with the newer SCF, MGDF, and G-CSF growth factor regimen, which has similar myeloid but significantly higher megakaryocyte expansion compared to the SCF, IL3, IL6, and G-CSF regimen

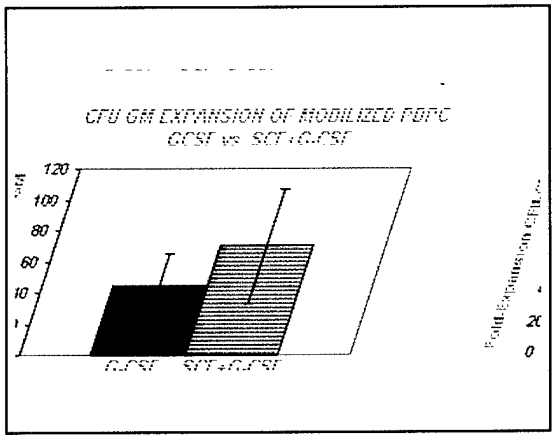


Figure 2

Effect of the Mobilization Regimen on the Ex Vivo Expansion of $CD34^+$ PBPCs. Our initial ex vivo expansion experiments used PBPCS mobilized with G-CSF alone. Figure 2 summarizes preliminary experiments using PBPCs mobilized with G-CSF alone (n=7) or SCF + G-CSF (n=7). The $CD34^+$ cells were isolated from the PBPC fractions; 1×10^4 cells/ml were cultured with ADM, SCF, G-CSF, IL-3, and IL-6. The results suggest that the S+G-CSF combination may mobilize PBPCs that are more responsive to ex vivo expansion ($p=0.05$). The current clinical study will attempt to confirm this preliminary result.

Ex Vivo Expansion: Baboon Studies. Recent studies performed by Drs. I. McNiece and B. Andrews in normal baboons demonstrated the potential clinical benefit of ex vivo expanded cells.[42] They harvested PBPCs from G-CSF mobilized normal baboons and expanded the $CD34^+$ cells for 10 days in SCF plus G-CSF plus MGDF. After the culture period, the cells were washed and infused into the baboons following lethal irradiation. GM-CFC were expanded 7-8 fold. Table 2 summarizes the engraftment characteristics of the different treatment groups in these studies. Despite the modest fold GM-CFC expansion (which may be species-dependant), Group IV, who received expanded $CD34^+$ cells, as well as posttransplant G-CSF and MGDF, had a significantly shorter duration of neutropenia and significantly higher WBC and PMN nadirs, compared to animals in the other groups. In fact, two of the three animals had no days with neutrophils below 500/μl, a clinical endpoint used for neutrophil engraftment. Additional studies to identify conditions which might impact platelet engraftment rates in this model are in progress.

Table 2. Engraftment of Irradiated Baboons with Ex Vivo Expanded PBPCs

Group (N=3)	In Vitro Expansion	Posttransplant G-CSF+MGDF	Days ANC >500/ul	ANC >1,000/ul	Platelets >1,000/ul	Nadir ANC(/ul)
I	No	No	7/16/16	15/28/26	14/8/26	9+16
II	Yes	No	13/17/12	24/70/21	NR/42/34	72+47
III	No	Yes	6/8/16	10/13/22	9/17/NR	50+40
IV	Yes	Yes	8/0/0	17/9/8	30/25/22	461+154

Clinical Expansion Studies. Several investigators have infused expanded progenitors to supplement an unmanipulated hematopoietic cell graft, in attempt to hasten engraftment. No toxicity relating to the infusion of cultured cells was noted. Engraftment rates appeared similar to historical controls that received unmanipulated hematopoietic cells alone.[43-45]

Cultured Cells as Sole Hematopoietic Support. A concern with using expanded progenitors as sole hematopoietic support is that the culture conditions could theoretically differentiate or Aexhaust≅ the stem cell compartment with long-term repopulating potential. Patients could experience not only rapid short-term engraftment, due to the infusion of expanded committed progenitors, but also delayed graft failure, resulting from the loss of primitive stem cells. Holyoake et al., reported that one of two patients, who received cultured PBPCs alone, did not engraft by day +14 and received an unmanipulated back-up fraction (which ultimately engrafted).[46] The culture conditions and CFU-GM content of their expanded grafts was suboptimal and make this negative study difficult to interpret. Brugger et al.,[44,47] conducted a pilot trial with the moderately intensive (not myeloablative) ifosfamide/carboplatin/etoposide (ICE) regimen, and 10% of a PBPC product, which was CD34 selected and expanded ex vivo, prior to infusion. Engraftment rates were identical to historical control patients who received the same preparative regimen and entire uncultured $CD34^+$ PBPC product. The problem with interpreting their study is that cellular support (unmanipulated or expanded) is probably unnecessary for rapid recovery of marrow with this non-myeloablative regimen.

The results of these early expansion studies were disappointing. In contrast, the baboon study, described above, demonstrated a significant reduction in in neutropenia using the same culture conditions we are proposing to use in the current clinical study. More recently, Stiff et al., cultured small bone marrow aliquots (median 30 ml) for 12 days in an Aastrom bioreactor and infused them as the sole source of hematopoietic support into 19 breast cancer patients who had received high-dose therapy.[37] Time to neutrophil and platelet engraftment was 18 days (range 13-22) and 24 days (range 19-27) respectively, which is similar to their historical controls who received a standard unmanipulated marrow autograft. At a median follow-up of more than one year, no patients had experienced myelosuppression or graft failure, even though more than half the patients received posttransplant radiotherapy. Given the caveat that one cannot rule out endogenous marrow recovery in this setting, these data are encouraging with respect to preserving progenitors with (relatively) long-term repopulating potential, using this bioreactor for culture

III. PATIENT ELIGIBILITY

Female patients with operable, histologically confirmed stage II/III or IV adenocarcinoma of the breast, enrolled on CU BMTP studies are eligible for this study.

IV. PRE AND POSTTRANSPLANT PATIENT EVALUATION

X Chest x-ray
X Bilateral bone marrow aspirates and biopsies (routine H&E staining)
X Radionuclide bone scan
X CT scans head, chest, abdomen, and pelvis
X Complete blood count
X Liver chemistry panel
X Creatinine clearance $\geq$ 60 ml/min.
X Pulmonary function tests with diffusion capacity $\geq$ 60% of predicted
X EKG and resting MUGA within normal institutional normal limits

V. TREATMENT PLAN

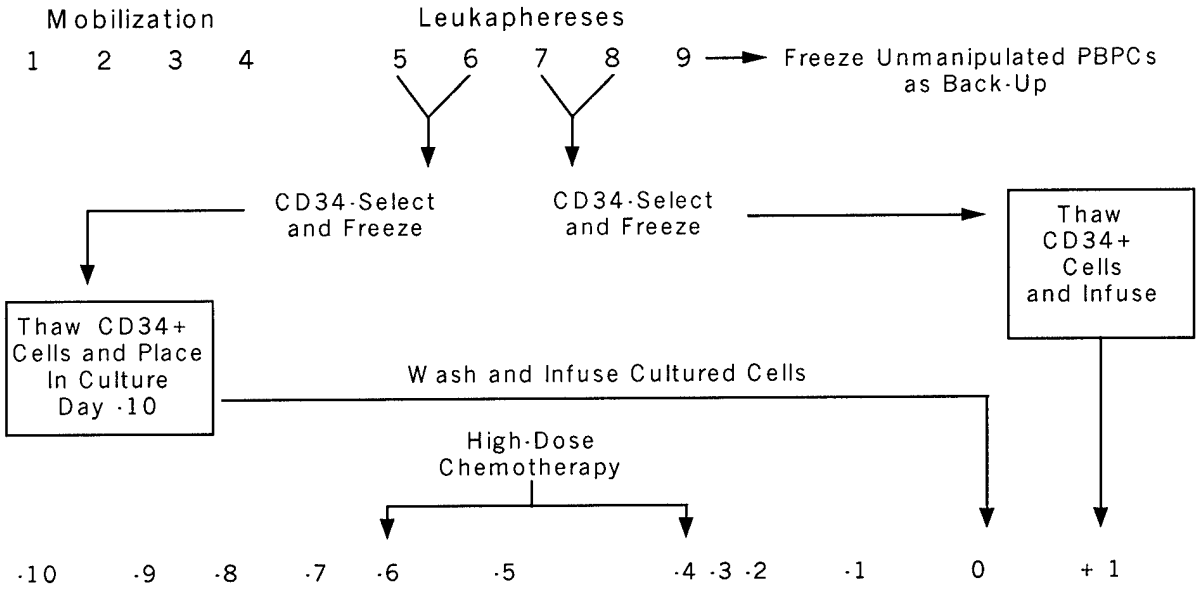
A. Study Design

This phase I/II trial will assess the impact of PBPC CD34 selection and ex vivo expansion on (1) time to engraftment and (2) the magnitude of breast cancer cell depletion from the PBPC autografts.

Patients who are receiving SCF plus G-CSF or G-CSF mobilization regimens will be entered into Cohort 1, where patients will receive $CD34^+$ PBPCs, a fraction of which have been expanded ex vivo. If adequate engraftment is demonstrated in the Cohort 1 patients, the study will progress to Cohort 2, where the patients will receive expanded PBPCs as the sole hematopoietic support.

Cohort 1: CD34-Selection and Ex Vivo Expansion. Growth factor(s) (G-CSF alone or G-CSF plus SCF) will be administered daily and leukaphereses will be performed on consecutive days 5, 6, 7, 8, and 9. The day 5 and 6 PBPCs will be combined, CD34-selected, and cryopreserved on day 6. The day 7 and 8 PBPCs will be combined, CD34-selected, and cryopreserved on day 8. The combined $CD34^+$ PBPC product (5/6 or 7/8) with the highest number of $CD34^+$ PBPCs will be cultured ex vivo, as described below, provided that the other combined product contains >1.0 x 10^6/kg CD34-selected PBPCs. If only one of the combined products contains >1.0 x 10^6/kg CD34-selected PBPCs, additional phereses will be performed to obtain the desired number of $CD34^+$ cells. If neither combined product contains >1.0 x 10^6/kg CD34-selected PBPCs, the patient will be removed from study and receive both $CD34^+$ and unmanipulated PBPCs per institutional protocol. Once the requisite number of $CD34^+$ cells are obtained, a back-up PBPC fraction will be collected and cryopreserved.

Ex Vivo Expansion of Growth Factor Mobilized PBPCs
Cohort 1

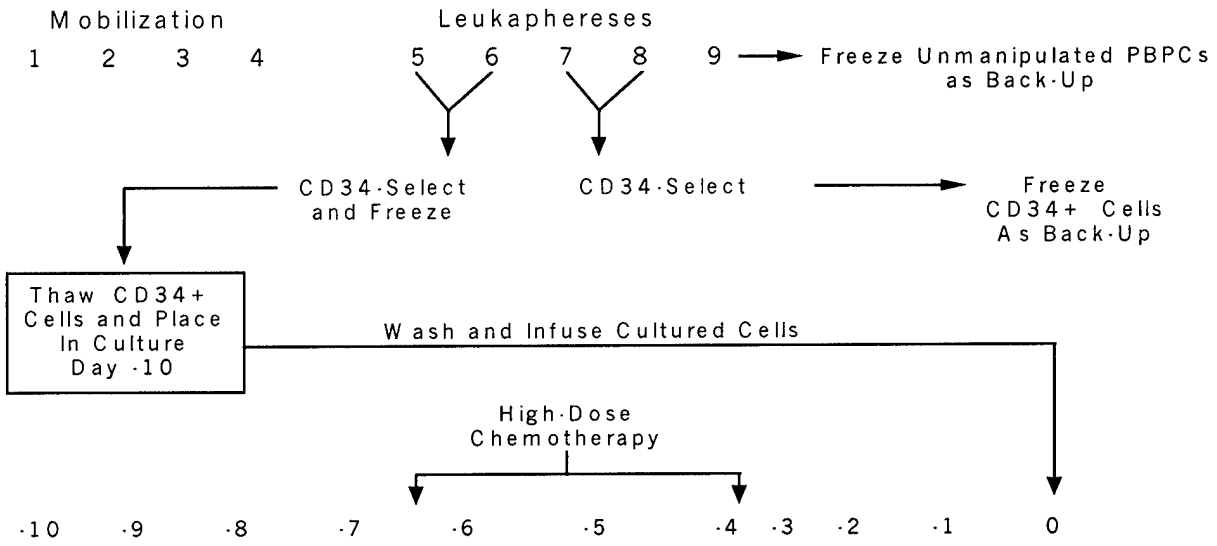


On chemotherapy day -10, the designated combined $CD34^+$ PBPC product will be thawed and placed into culture with defined media plus SCF, MGDF, and G-CSF (AMGEN, Inc) in a concentration of 5 x 10^4 or 1.0 x 10^5 cells/ml. On days -6 through

-3, high-dose therapy will be administered. On day 0, (which will be ex vivo culture day +10), the cultured PBPCs will be washed and infused. On day +1, the other frozen CD34-selected PBPC product will be thawed and infused. Systemic G-CSF will be administered daily from day 0 (following PBPC infusion) until an ANC of 5.0 x 10^9/L for two consequetive days or an ANC of 10.0 x 10^9/L is documented.

Cohort 2: Expanded PBPCs as Sole Hematopoietic Support. Growth factor(s) (G-CSF plus SCF or G-CSF alone) will be administered daily and leukaphereses will be performed on consecutive days 5, 6, 7, 8, and 9. The day 5 and 6 PBPCs will be combined, CD34 selected, and cryopreserved on day 6. The day 7 and 8 PBPCs will be combined, CD34-selected, and cryopreserved on day 8. The combined $CD34^+$ PBPC product (5/6 or 7/8) with the highest number of $CD34^+$ PBPCs will be cultured ex vivo, as described below, provided that the other combined product contains >1.0 x 10^6/kg CD34-selected PBPCs. If only one of the combined products contains >1.0 x 10^6/kg CD34-selected PBPCs, additional phereses will be performed to obtain the desired number of $CD34^+$ cells. If neither combined product contains >1.0 x 10^6/kg CD34-selected PBPCs, the patient will be removed from study and receive both $CD34^+$ and unmanipulated PBPCs per institutional protocol. Once the requisite number of $CD34^+$ cells are obtained, a back-up PBPC fraction will be collected and cryopreserved.

Ex Vivo Expansion of Growth Factor Mobilized PBPCs
Cohort 2



On chemotherapy day -10, the designated combined $CD34^+$ PBPC product will be thawed and placed into culture with defined media plus SCF, MGDF, and G-CSF (AMGEN Inc) in a concentration of 5.0 x 10^4 or 1.0 x 10^5 cells/ml. On days -6 through -3, high-dose therapy will be administered. On day 0, (which will be ex vivo culture day +10), the cultured PBPCs will be washed and infused.

B. Study End-Points

The primary clinical end-points of the study include assessment of the infusional toxicity produced by the expanded PBPC progenitors, as well as the number of days (from day 0) to achieve neutrophil and platelet engraftment, as defined below. Secondary end-points include disease-free and overall survival.

1. **Evaluation of Primary End-Point: Infusional Toxicity.** Patients enrolled on this study will be evaluated before and after the infusion of expanded PBPCs, using the parameters and schedule outlined in Table 3. The clinical and laboratory evidence of toxicity will be recorded on the Infusional Toxicity Flowsheet included in Appendix C.

2. **Evaluation of Primary End-Point: Engraftment.** In this study, neutrophil engraftment is defined as a sustained ANC $\geq 0.5 \times 10^9$/L, and platelet engraftment as a sustained transfusion-independent platelet count $\geq 20 \times 10^9$/L for three consecutive days, without transfusion support.

   a. Engraftment Failure. Engraftment failure will be defined as failure to achieve an ANC $\geq 0.5 \times 10^9$/L by day 28 and/or failure to achieve a transfusion-independent platelet count $\geq 10 \times 10^9$/L by day +100 posttransplant. Patients experiencing platelet engraftment failure must have no evidence of autoimmune thrombocytopenia and bone marrow biopsies demonstrating decreased numbers of megakaryocytes. If either neutrophil or platelet engraftment failure is documented on the days specified above, a back-up hematopoietic cell fraction will be infused.

   b. Late Graft Failure. Late graft failure will be defined as the development of clinically significant neutropenia (ANC $\leq 0.5 \times 10^9$/L) or thrombocytopenia (transfusion-dependant platelet count $\leq 10 \times 10^9$/L) at two months posttransplant or later, after having demonstrated adequate engraftment in the early posttransplant period. If this occurs and does not resolve with standard medical management including growth factor support, the unmanipulated back-up hematopoietic cell fraction will then be infused.

Table 3. Parameters Followed to Assess the Infusion-Related Toxicity of Expanded PBPCs

Parameters	Preinfusion	During and Postinfusion	24 Hours Postinfusion

Temperature	+	+ (a)	+
Blood Pressure	+	+ (a)	+
Pulse Rate	+	+ (a)	+
Respiratory Rate	+	+ (a)	+
Pulse Oximetry	+	+ (a)	+
Cardiac Rhythm Strip	+	+ (a)	+
Other Systemic Toxicity (b)	+	+	+
CBC, Platelets, Differential	+	-	+
Serum Chemistries (c)	+	-	+
Urinalysis (dipstick) (d)	+	+	+
Antiemetic Record (e)	+	+	+

(a) Measured every 15 minutes after the initiation of the infusion for one hour and then at two hours, four hours, and 24 hours postinfusion.

(b) Specific organ toxicity as defined in Appendix C will be recorded preinfusion and during the first 24 hours postinfusion.

(c) Includes electrolytes, BUN, creatinine, SGOT, SGPT, alkaline phosphatase, and bilirubin; measured preinfusion and 24 hours postinfusion.

(d) Urinalysis will be performed and recorded preinfusion and on every urine specimen obtained during first 24 hours postinfusion; if patient catheterized, a urinalysis should be performed once every four hours.

(e) All antiemetics administered will be recorded for 24 hours before and after the infusion of expanded PBPCs.

3. **Statistical Analysis of Primary End-Points**. Fifteen patients will be accrued to each cohort. These two cohorts will be treated within 2.5 years. Each cohort evaluation will be completed before the next evaluation is initiated. Based on the results from our previous studies which resulted in a mean time of engraftment of ANC of 10 days, range 8-15, and SD=1.75 and of platelets of 11 days, range 8-14, SD=1.5, we have 98% power with 15 patients/cohort and 91% with 10 patients/cohort to detect a difference of 2-3 days in time to engraftment (calculated using SoloPower). Our detectability limits for engraftment are 8-12 days for ANC and 9-20 days for platelets with no more than 25% failure.

a. <u>Early Stopping Rule for Infusion-Related Toxicities and Engraftment Failure</u>. A three-stage stopping rule for serious (grade 4) infusion-related toxicity or engraftment failure (defined above) will be employed. The two primary end-points will be tracked separately. If three or more grade 4 toxicities are observed in the first 10 patients,

the trial will terminate and we will conclude that the rate of serious infusion-related toxicity or engraftment failure is unacceptably high. If fewer than three grade 4 infusion-related toxicities or engraftment failures occurs, an additional 10 patients will be enrolled and observed; if the cumulative number of grade 4 toxicities is greater than or equal to 4, then the trial will terminate as above. This design has 86% power to stop the trial if the true rate of grade 4 infusion-related toxicity or engraftment failure exceeds 20%. The trial has less than a 1% probability of being terminated, however, even when the grade 4 infusion-related toxicity or engraftment failure is 5%. A 95% confidence interval about the observed grade 4 toxicity rate will be calculated at the end of the trial.

4. **Statistical Analysis of Secondary End-Points**. The secondary end-points of disease-free and overall survival will be evaluated using Kaplan-Meier Life Table Analyses.

C. Food and Drug Administration (FDA) Approval

This study will be conducted under BB-IND #7055 as assigned by the FDA in May, 1998.

D. Reporting Procedures

All adverse events occurring during the treatment phase will be recorded. All serious adverse events occurring curing the treatment phase and up to day 60 of the follow-up (irrespective of the suspected causation) will be recorded and evaluated. A copy of all correspondence with the FDA regarding serious adverse events will be sent to Amgen Inc and Baxter Inc.

VI. HEMATOPOIETIC CELL COLLECTION AND PROCESSING PROCEDURES

A. Selection of $CD34^+$ PBPCs

$CD34^+$ cells will be isolated from PBPCs using the Baxter Isolex9 300 System. All procedures will be performed using aseptic technique. The selection procedure includes specific steps for:

1. PBPC Preparation. A platelet wash is performed on the apheresis product, prior to the isolation procedure. This occurs using the spinning membrane assembly, which is part of the disposable set (wash chamber).
2. Sensitization of the mononuclear cells with the 9069 anti-CD34 monoclonal antibody. This step is performed with 2.5 mg of anti-CD34 antibody in a total volume of 100 mL. Sensitization is performed for fifteen minutes at room temperature, after which the cells are washed to remove excess/unbound antibody.

3. Rosetting of target cells and paramagnetic beads. Freshly prepared Dynal7 paramagnetic microspheres (SAM IgG) are then added to the washed, sensitized cells. After incubation at room temperature, the bead-cell complexes are separated from the unbound cells using the primary magnet. The bead/target cell complexes are retained in the separation chamber and the nontarget cells are washed away.
4. Release of target cells from the beads. Using the stem cell releasing agent, the $CD34^+$ cells are displaced, releasing them from the beads. This requires incubation at room temperature, after which the target cells are collected, using the primary magnet to hold the beads in the chamber.
5. Washing of isolated $CD34^+$ cells.

B. Ex Vivo Expansion of Thawed $CD34^+$ PBPCs

The CD34-selected PBPCs will be cultured in one liter teflon-coated bags (American Fluoroseal) containing defined media and 100 ng/ml of SCF, MGDF, and G-CSF (Amgen) for 10 days. The cultures will be inoculated with 5×10^4 or 1.0×10^5 $CD34^+$ cells/ml and maintained at 37EC, 5% CO_2, and 95% humidity.

C. Washing and Infusion of Expanded PBPCs Following Culture

1. **Culture Completion.** After 10 days in culture, the cells will be washed on the COBE 2991 with 2-3L of D-PBS; the Ex-Vivo Cell Processing bag containing the cultured cells will be hung on the 2991 which will have been preloaded with a single processing set. Once the cells and PBS enter the centrifuge bowl, they will be spun at 3000 rpm for five minutes; the supernatant is expressed and the entire process repeated for a total of three volume exchanges. Aliquots from each supernatant will be collected for cytokine level analysis and aliquots will be sent for bacterial, fungal, and mycoplasma culture.

2. **Release Criteria.** The product will only be released for infusion if the visual inspection is within normal limits, the stat gram stain is negative, and the viability is greater than 70%.

D. PBPC Autograft Evaluation

The selected and expanded PBPC cells will be evaluated phenotypically using the flow cytometric and immunohistochemical assays described below, and functionally in vitro with the CFU-GM assay.

1. **Flow Cytometric Evaluation: Phenotypic Characterization of PBPCs.** Red cells will be lysed with ammonium chloride. Propidium iodide (Sigma) will be used to detect nonviable cells. 5×10^5 cells per sample will be stained with the primary antibody of interest. A consistent panel will be used for the

expanded PBPC cells, including anti-CD34 [Beckton-Dickenson (B-D)]; lymphoid-specific: CD3, CD4, CD8, CD19, and CD10 (Coulter). Two-color flow cytometric analysis will be performed with an EPICS XL flow cytometer and the software provided (Coulter Inc., Hialeah, FL).

2. **Short Term Colony Forming Cell Assay for PBPC Myeloid and Erythroid Progenitors.** The PBPC progenitors will be placed in Methylcellulose culture media (MethoCult GF^+ (H4535), Stem Cell Technologies Inc., Vancouver, B.C.) in a laminar air flow hood using sterile technique. Seeding densities of 0.1 - 5 $x10^4$ will be employed. The media includes 0.9% Methylcellulose in Iscove=s MDM, 30% Fetal Bovine Serum, 1% Bovine Serum Albumin, 10^{-4}M 2-Mercaptoethanol, 2mM L-glutamine, 50ng/ml Stem Cell Factor, 20ng/ml GM-CSF, 20ng/ml IL-3, 20ng/ml IL-6, and 20ng/ml G-CSF. All samples will plated in triplicate in 35x10mm gridded dishes (NUNC, Naperville, IL) both with and without 5 units/ml Erythropoietin (EPO, Amgen, Thousand Oaks, CA). Cultures will be maintained at 37°C, 5% CO_2, and 95% humidity. After 14 days the plates are scored for colony forming cells-Granulocyte Macrophage (CFU-GM) and Burst forming unit erythroid (BFU-e) colonies using an inverted phase microscope.

3. **Immunostaining of Megakaryocyte Progenitors.** The ficoll-prepared mononuclear cell (MNC), $CD34^+$, and cultured PBPC cells of interest will be washed once in TC-199 medium containing 20% fetal calf serum and diluted to a concentration of 25 x 10^6 cells/ml. Two hundred µl of this cell suspension is added to a cytocentrifuge slide holder which is preloaded with silane coated glass slide and a number 2 filter paper. Cells are centrifuged at 500 rpm for 5 minutes, air dried for 30 minutes, and fixed for 20 minutes in a suspension of 45% methanol, 45% acetone and 10% concentrated (37%) formaldehyde.

   The fixed slides are incubated sequentially with the primary mouse anti-megakaryocyte antibody CD41 for one hour followed by a rabbit anti-mouse IgG for 30 minutes, and finally with a complex composed of alkaline phosphatase and monoclonal mouse anti-alkaline phosphatase for one hour. The slides are washed three times with Tris buffer in a lucite tank between each of the three antibody incubations. After the final Tris wash, the slides are incubated for 30 minutes with New Fuchsin substrate containing 0.35 mg/ml levamisole to suppress endogenous alkaline phosphatase activity. The cells are then lightly counter-stained with hematoxylin. Stained slides are examined using a standard binocular light microscope with a low power (10x) objective.

4. **Breast Cancer Cell Assays.**

a. Immunohistochemical Analysis. Cytocentrifuge slides will be prepared using a Cyto-Tek (Miles Scientific, Elkhart, IN). Two hundred µl of cell suspension containing $5x10^5$/ml will be added to the cytocentrifuge slide holder loaded with a silane-coated glass slide and number 1 cc filter paper. Cells are then centrifuged onto the slide at 500 rpm (160 x g) for 5 minutes, air dried for 30 minutes, and fixed with acetone/methanol/formalin (45%/45%/10%). Fixed cells are stained in 200 ml containers according to a modification of the APAAP technique [1] and are counterstained with hematoxylin. Stained slides are examined using a standard binocular light microscope with a low power (10X) objective. The number of tumor cells detected per 10^6 bone marrow cells in each replicate assay will be regressed on the number of tumor cells added per 10^6 marrow and/or PBPCs, and a quantitative assessment of tumor cell content calculted.

b. RT-PCR Assay for CK-19 and muc-1. Total RNA will be purified from the marrow and PBPC specimens. Reverse transcriptase wiil then be used to make cDNA from the RNA. The cDNA will be heated to 95°C. at which point it will dissociate into single strands (Denaturation Step). The reaction mixture will then be cooled which stimulates the single-stranded DNA to hybridize to two single-stranded oligonucleotide primers that flank that DNA. A substantial excess of the CK-19 or muc-1 primers will be added to the cooling suspension; the primer will then anneal to the template rather than it's complementary strand (Annealing Step). Taq polymerase will then incorporate deoxyribonucleotide triphosphates onto the 3' end of the primers. Reading the template DNA strand in a 3'-->5' direction Taq polymerase inserts complementary bases into the new, elongating DNA strand in a 5'-->3' direction. The synthesized copies will become the templates for the next cycle with the result that, with each cycle, there is a geometric increase in the number of templates in the reaction (Elongation Step). After 30-50 cycles of the PCR, CK-19 or muc-1 will be detected by ethidium bromide staining and observation of its characteristic mobility in agarose gels.

VII. FORMULATION OF REAGENTS USED IN EXPANSION PROCEDURE

See Protocol #96-154 for the drug information pertaining to the cytoreductive and GVHD prophylaxis/treatment regimens. The $CD34^+$ PBPCs will be cultured with very low concentrations of the growth factors described in this section (nanogram concentrations as opposed to the microgram concentrations used systemically). Additionally, the expanded

cells will be washed three times prior to infusion into the patient, which eliminates all detectable levels of the growth factors. Thus, it is unlikely that they will produce any systemic side effects.

A. Recombinant-metHuStem Cell Factor (SCF)

Recombinant methionyl human stem cell factor (r-metHuSCF) is a recombinant human protein produced in E. coli by recombinant DNA technology. The 165-amino acid non-glycosylated protein contains two intramolecular disulfide bonds, exists as a non-covalently associated dimer with a molecular weight of 36,000, and differs from the natural protein by the presence of a methionine moiety at the N-terminus (residue number [-1]) resulting from the expression in and in the fact that the recombinant protein is not glycosylated. Cells expressing r-metHuSCF are grown in culture under defined and controlled conditions. The cells are harvested yielding a paste from which the r-metHuSCF is extracted and purified via a series of proprietary processing and chromatographic steps. The resulting purified r-metHuSCF is formulated in an aqueous buffer before undergoing sterile filtration and filling. Criteria for release of r-metHuSCF for use in the clinic are stringent. These include passing the USP rabbit pyrogen test, the limulus amebocyte assay, a sterility test, and the general safety test (Code of Federal Regulations, Title 21, Section 610.11). The nucleic acid content is no greater than 1.7 pg/mg protein. The final product is a clear, colorless, sterile protein solution free of particulates; r-metHuSCF is not less than 95% pure. Biologic activity of purified preparations is assessed via radioreceptor binding and proliferation assays.

1. **Supply and Return of Drug**. The r-metHuSCF will be shipped to the University of Colorado under the name of the Dr. Shpall at study initiation and as needed thereafter. If the drug is rendered unusable during transit due to temperature extremes, breakage, etc., replacement material will be provided. At the end of the study or as directed, all unused vials will be returned to Amgen Inc. in the original shipping box. Used vials will be destroyed on site using approved methods of destruction or returned to Amgen Inc., as directed.

2. **Storage.** Recombinant-metHuSCF must be stored at 2-8EC. Stability of r-metHuSCF at concentrations of 1.5 mg/ml has been demonstrated for 12 months when stored under these conditions. Stability testing is ongoing. Exposure of the material to excessive temperatures above or below this range is to be avoided. Do not allow r-metHuSCF to freeze, and do not use if contents freeze in transit or in storage.

B. Recombinant-met Granulocyte Colony Stimulating Factor (G-CSF)

1. **Supply and Return of Drug.** The r-metHuG-CSF will be shipped to the

University of Colorado under the name of the Dr. Shpall at study initiation and as needed thereafter. If the drug is rendered unusable during transit due to temperature extremes, breakage, etc., replacement material will be provided. At the end of the study or as directed, all unused vials will be returned to Amgen Inc. in the original shipping box. Used vials will be destroyed on site using approved methods of destruction or returned to Amgen Inc.

2. **Storage.** Recombinant-metHuG-CSF must be stored at 2-8EC. Stability of r-metHuG-CSF at concentrations of 0.30 mg/ml has been demonstrated for 24 months when stored under these conditions. Exposure of the material to excessive temperatures above or below this range is to be avoided. However, if product is inadvertently frozen for up to 24 hours, it can be used.

C. Recombinant-met Megakaryocyte Growth and Differentiation Factor (MGDF)

1. **Supply and Return of Drug.** The r-metHuMGDF will be shipped to the University of Colorado under the name of the Dr. Shpall at study initiation and as needed thereafter. If the drug is rendered unusable during transit due to temperature extremes, breakage, etc., replacement material will be provided. At the end of the study or as directed, all unused vials will be returned to Amgen Inc. in the original shipping box. Used vials will be destroyed on site using approved methods of destruction or returned to Amgen Inc.

2. **Storage.** Recombinant-metHuMGDF must be stored at 2-8EC. Stability of r-metHuMGDF at concentrations of 1.5 mg/ml has been demonstrated for 12 months when stored under these conditions. Stability testing is ongoing. Exposure of the material to excessive temperatures above or below this range is to be avoided. Do not allow r-metHuMGDF to freeze, and do not use if contents freeze in transit or in storage.

D. Isolex7 300-i Cell Selector Device

The sterile, disposable CD34-selection columns and tubing sets will be supplied by Baxter Healthcare Corporation.

1. **Isolex7 300i Disposable Tubing Sets.** Single-usage prepackaged sterile tubing set.

2. **Isolex7 300 Reagent Kit and Disposable Tubing Set Return.** The packages of CD34-selection reagent kits and disposable tubing sets will be supplied by Baxter Healthcare Corporation. They will be shipped to the University of Colorado under the name of the Dr. Shpall at study initiation

and as needed thereafter. If the reagent kits and/or tubing are rendered unusable during transit due to temperature extremes, breakage, etc., replacement material will be provided. At the end of the study or as directed, all unused reagents and disposable tubing sets will be returned to Baxter Healthcare Corporation as directed.

3. **Storage.** The packages of CD34-selection columns and disposable tubing sets must be stored at 2-8EC. Stability of columns and tubing sets have been demonstrated for 12 months when stored under these conditions. Stability testing is ongoing. Exposure of the material to excessive temperatures above or below this range is to be avoided. Do not allow the columns and/or tubing to freeze, and do not use if contents freeze in transit or in storage.

E. Tissue Culture Bags Employed in Culture Expansion

1. **Supply and Return of Tissue Culture Bags.** The tissue culture bags used for the expansion cultures will be obtained from American Fluoroseal, Inc.

2. **Storage.** Tissue culture bags should be stored at room temperature. Exposure of the material to excessive temperatures above or below this range is to be avoided. Do not allow the bags to freeze, and do not use if contents freeze in transit or in storage.

F. *CLINI*MACS CD34$^+$ Cell Selection

The *Clini*MACS is comprosed of four primary components.

(a) *Clini*MACS Instrument: A software controlled instrument that process the the cell sample.
(b) *Clini*MACS Tubing Set: A single-use, sterile disposable tubing set with two proprietary cell selection columns.
(c) *Clini*MACS CD34 Reagent: An iron/dextran colloid conjugated to a monoclonal antibody magnetic bead specific for CD34+ cells.
(d) *Clini*MACS PBS/EDTA Buffer: A sterile, isotonic, phosphate buffered, 1mM EDTA, saline solution used as external wash and transport fluid for the *in vitro* preparation of blood cells.

A brief description of each component is provided below.

CliniMACS Instrument

The *Clini*MACS Instrument is a bench-top instrument consisting of a supporting structure to hold the column/tubing assembly and various bags, a series of control

valves through which the tubing set is fitted, a movable magnet, a peristaltic pump, a liquid sensor, software and a computer touch pad with a display window. The instrument is operated at ambient temperature and it is intended to be a multi-use item. Photographs of the *Clini*MACS Instrument and Instrument Tubing Set Mounted are provided in Figures 1 through 3 below.

The software for the *Clini*MACS Instrument controls the function of the electromechanical components of the instrument and the user interface. Two separate computers, one a micro-controller located on a control board inside the *Clini*MACS and a PC compatible computer that operates the user interface are incorporated into the instrument. The instrument is supplied with software version 2.21, which is directly traceable to the version of software utilized I pre-clinical testing and clinical trials, and has been inspected and approved by TUV product services with the CE Mark. Version 2.21 is also directly traceable to software version 2.10, which has been marketed in the European Community (EC) since December 1997. The software is Y2K compliant.

CliniMACS Tubing Set

The *Clini*MACS Tubing Set consists of tubing, bags and a pair of proprietary cell selection columns. The separation column is a proprietary component of the *Clini*MACS System consisting of a plastic column housing with polypropylene frits at each end (See Figure 4). The interior of the column housing is filled with a matrix of sub-millimeter iron beads coated with a heat-cured resin. The columns are placed at appropriate locations in the *Clini*MACS Tubing Set to facilitate the cell selection process. The first column serves as a device to remove components from the stained cell mixture that may bind non-specifically to the column. The second column performs the actual cell selection. The columns are provided sterile as part of the tubing set and are intended for single use only.

The balance of the tubing set consists of a series of tubes, connectors, spikes, Luer locks, and collection bags. The tubing is comprised of materials that have been qualified for use by testing to EN ISO 10993-1. The principal constituents are polyvinyl chloride (PVC) and silicone. The connectors are made of various polymers (ABS and PVC). These are solvent bonded to the PVC tubing with cyclohexanone. The silicone pump tubing is softened with petroleum ether for manufacturing and mechanically fixed to connectors. The bags are composed of PVC and are intended for blood collection and storage.

The *Clini*MACS Tubing Set is packaged in a thermoform tray and heat sealed with a Tyvek® lid. The *Clini*MACS Tubing Set is sterilized by ethylene oxide gas and supplied as a single-use component for the *Clini*MACS Instrument. The sterilized set has been tested to conform to harmonized bio-compatibility standard (EN ISO

10993) for a limited duration direct contact device.

CliniMACS CD34 Reagent

The *Clini*MACS CD34 Reagent is a dark amber, nonviscous, colloidal solution containing the antibody conjugate in buffer. The conjugate consists of a murine monoclonal antibody (to CD34 antigen) covalently linked to dextran beads having an iron oxide/hydroxide core. The concentration of the conjugate is equivalent to 22 ug of antibody protein per mL of reagent, 800ug/Ml of dextran and 800ug/mL of iron.
The colloid is buffered in a phosphate-buffered saline (PBS) containing ethylenediaminetetraaceetic acid (EDTA) and Poloxamer 188. The nominal concentrations of its components are 0.0095 M phosphate, 0.004 M potassium, 0.163 M sodium, 0.139 M clorine, 0.005 M EDTA and 0.03% (w/v) Poloxamer 188. The pH is 7.4-7.7

Poloxamer 188 is added to the *Clini*MACS CD34 Reagent to stabilize it during shipping, handling, and storage. The *Clini*MACS CD 34 Reagent is supplied sterile and pyrogen-free in glass vials containing 7.5mL and is intended for single use and *in vitro* use only.

The CliniMACS PBS/EDTA Buffer

The *CLINI*MACS PBS/EDTA Buffer is and isotonic and isohydric buffer solution with a Ph-value of 7.2 and osmolarity of 290 mosmol/L. Its formulation is shown in the following table.

Table 5.

Ingredient	Compendial	Amount
NaCl	Ph. Eur.	8.0 g/L
KCl	Ph. Eur.	0.19 g/L
Na_2HPO_4anhy.	Ph. Eur.	1.15 g/L
KH_2PO_4	Ph. Eur.	0.19 g/L
Na_2EDTA	Ph. Eur.	0.37 g/L
Water for Injection	Ph. Eur.	Ad 1L

The *Clini*MACS PBS/EDTA Buffer is used as external wash and transport fluid for the *in vitro* preparation of human leukapheresis products intended to be separated with the *Clini*MACS Cell Selection System.

This is achieved by the following procedure:

Cells for separation are obtained by leukapheresis. After incubating the cells with CD34 Reagent in PBS/EDTA Buffer supplemented with 0.5% human serum albumin (HAS), the excess of unbound reagent is removed by washing with the PBS/EDTA Buffer + HAS. During the following automated cell selection process, using PBS/EDTA + HAS the unwanted cells are removed and in the final step, the selected cells are eluted from the column by the means of the PBS/EDTA Buffer. The HAS is not part of the buffer, but is added at the clinical site.

Principles of Operation

The function of the *Clini*MACS System is to highly enrich CD34+ cells from heterogeneous cell populations in the patient's leukapheresis/apheresis product. The cell selection process involves two phases; cell labeling prior to selection (phase I) and the automated cell selection process (phase 2).

Phase I

This phase is referred to as the cell labeling step. This involves combining the antibody reagent (conjugated with iron-dextran) and the patient's apheresis product. The antibody reagent is incubated for 30 minutes with the heterogeneous cell population during which the antibody selectively binds to cells expressing the CD 34 antigen. This mixture is then washed twice in PBS/EDTA buffer supplemented with 0.5% HAS. Following centrifugation, the resulting cell pellet is resuspended I the depatation buffer and the labeled product is ready for cell selection.

Phase II

The labeled cell sample is attached to the *Clini*MACS System. Following a series of automated priming steps, the cell suspension is passed through a 40 um mesh blood filter (Pall Medical) to remove any cell aggregates that may be present. The cell suspension is then passed through the first column, which serves as a pre-column separation step to reduce non-specific binding. The labeled sample then passes through the second column positioned within the magnetic field. The CD34+ cells, to which the antibody reagent has been bound, are selected and retained in the column matrix. All other calls flow through the column and are collected in the "negative fraction" bag.

After automated buffer washed of the column containing the isolated CD34+ cells, it is removed from the magnetic field, and the CD34+ cells are eluted into the "positive fraction" bag. The selected cells can be used immediately or cryopreserved for later infusion into the patient.

The device is non-invasive in all aspects that involve processing of the apheresis product. Infusion of the selected cells is accomplished by cell bound iron-dextran conjugated antibody reagent (<0.6 ug protein/kg) and depending on the handling and freezing process after selection, small amounts of PBS with EDTA, HAS and DMSO may be present. All testing to date has revealed the level of exposure of reagent and buffer are considered safe and present no risk of harm to the patient.

VIII. CRITERIA FOR REMOVAL FROM STUDY

Any patient can be removed from study if, in the judgment of the Principal Investigators, further treatment is not in the best interest of the patient for whatever reason. Such patients will be scored as treatment failures at that moment for purposes of further follow-up and study reporting.

IX. DOSE MODIFICATIONS

Dose modification of the growth factors used for ex vivo expansion cultures will not be permitted.

X. Confidentiality and Security

Non-computer based protocol related data are stored in three locations:

1. University Hospital, 4 West Inpatient Unit.
2. University Hospital, 4 North Outpatient Clinic.
3. Administrative Office Building (AOB) of Bone Marrow Transplant Program, Administrative Office Room 252.

The use of protocol documents in the inpatient and outpatient clinic is under the constant supervision of nursing and administrative staff in those areas and is restricted to medical personnel who must participate in patient care. The inpatient unit documentation is under 24 hour a day supervision of the administrative and nursing staff of the unit. The outpatient documents are under such supervision during outpatient clinic hours and are maintained in a locked room otherwise. The AOB files are likewise maintained under the supervision of office staff and are in a locked room after hours.

Computer Data

Computer data are maintained in the Bone Marrow Transplant Program oracle database in file server structures. The computers themselves are located in a locked room, which is only available through a coded door lock to authorized Bone Marrow Transplant Program staff. Patient specific information is only available to authorized users through a three level password system. 1) Password at the workstation level, 2) password identifying the database product, 3) individual password, which limits

accessibility to specific areas based upon level of need of the personnel. Passwords at the third level are changed every three months. The use of the oracle database for primary patient storage ensures that field level protections can and are utilized to avoid export of any unauthorized data outside the Bone Marrow Transplant Program.

XI. Procedures for Obtaining Informed Consent

The following procedures are followed in the Bone Marrow Transplant Unit to obtain informed consent for any study.

Patients are only referred to the Bone Marrow Transplant Program through oncologists or other non-bone marrow transplant personnel who identify these patients as potential candidates. After referral and submission of detailed medical information patients are seen at the University Hospital or are using a Tele-medicine consult method. At that time, the consulting physician in the presence of a nurse coordinator provides detailed information concerning the proposed protocols (risks and benefits). After the physician leaves, the nurse coordinator reviews the available data with the patients. In addition, social worker staff, psychology staff, and financial consultants who all help in the overall consent process see the patient.

After the patient verbally agrees to undertake the proposed treatment they are provided with written consent documents outlining the IRB approved nature of the programs. The patients have these documents to take home and they are encouraged to call physicians and nurse coordinator staff to discuss any elements of the proposed consent, which are of concern to them.

After the patient returns to the University Hospital to begin the pre-transplant process a meeting lasting a minimum of one hour occurs with the patient, family or friends, a BMT nurse coordinator, and the attending BMT physician. At that time the consent is reviewed in detail with the patient. They are given ample opportunity to ask and have all of their questions answered concerning the proposed studies. If the patient and family or friends consider they have an adequate understanding of the proposed protocols they are allowed to sign at that time in the presence of the attending physician and nurse coordinator. If there is inadequate comprehension or doubt the patient is encouraged to take the consent form return home or to the hotel and review it in sufficient details to resolve these issues prior to signing the consent. After all the consents are signed the pre-transplant process is initiated. This involves testing and procurement of a bone marrow or stem cell product.

It is important to note that the attending physician is always a co-investigator on the protocols, and that the principle investigator is often selected at random from Bone Marrow Transplant Program staff in order to balance

protocol load appropriately.

After the patient is admitted to the hospital but before the transplant process begins Bone Marrow Transplant Unit staff nurses again review the nature (risks and benefits) of the treatment with the patient. Treatment does not proceed unless the nurses document in the medical record that it is their belief adequate consent has been given. In addition, the nurses are required to review the signed consent forms to be sure that all are in place in the medical record, and to review all of the medical orders pertaining to each of the protocols to ensure they are correct prior to the initiation of treatment.

Only after all of these procedures are done is the treatment process initiated.

NON-ENGLISH SPEAKING PATIENTS

While it is not the intent of this protocol to deliberately recruit non-English speaking patients it is assumed that they will be occasionally be enrolled on this study. In particular, in the greater Denver area Hispanic patient are the most likely persons in this category to be enrolled. When this happens, the following procedures will be used:

1. If any family member or close friend is fluent in English they will accompany the patient to all consent meetings with staff as described above.
2. At the meeting where the actual consent form will actually be signed, a Spanish speaking member of the BMT staff, who are completely knowledgeable about the protocol in question will perform the primary consent process. Specifically, they will explain in Spanish the rationale for the study, risks and potential benefits involved.
3. In addition, the BMTP will commit to make available a Spanish speaking interpreter for the patient on a daily basis to ensure ongoing consent and adequate information exchange.
4. In the case of non-English speaking individuals with other primary languages, a translator will be made available during the consent process to translate both the content of the consent form and information given from the BMT physician.

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XI. APPENDIX A. Worksheets and Procedures

EX-VIVO EXPANSION OF CD34 SELECTED PBPC

PRINCIPLE

CD34 selected PBPC will increase in total cell, total colony forming unit and total CD41 content when cultured for ten days in defined medium containing 100ng/ml of Stem Cell Factor, Granulocyte-Colony Stimulating Factor, and Megakaryocyte Growth and Differentiation Factor. By increasing the number of cells, colony forming units and CD41 cells available, the length of time to engraftment may be shortened. Ex-Vivo culture may also facilitate the use of smaller cord blood units for larger patients.

SPECIMEN

This procedure is intended for use with PBPC that have been CD34 selected following the CD34 Selection of PBPC Procedure in the Stem Cell Engineering Clinical Laboratory Procedure Manual.

MATERIALS

All the items listed in Appendix A: Laboratory Materials and Equipment List should be available. Additionally the following supplies (supplied by Amgen) are required:

Ex-Vivo Cell Processing Bag
Defined Medium
Cytokines: Stem Cell Factor (SCF)
Granulocyte Colony Stimulating Factor (G-CSF)
Megakaryocyte Growth and Differentiation Factor (MGDF)

PROCEDURE

Culture Initiation:

1. Perform a cell count and calculate the total number of cells available following CD34 selection. Calculate the number of cells that would be required to perform the following assays: %CD34/3, Colony Forming Unit Assay (CFU-GM), and 2 cytospins for WBC differential and CD41 by IHC. Multiply the total cells available by 10%. Perform the assays if the number of cells required is less than 10% of the cells available. The priority is as follows: CFU-GM, CD34/3, CD41.

2. Remove a 3 ml and a 5 ml aliquot of Defined Medium from the media supply bag=s sampling site using an 18g needle and 5 cc syringe.

3. Transfer the remaining Media into the Ex-Vivo Cell Processing bag using one of the spikes on the attached tubing set. Sebra seal and detach the line at the junction of the spike used.

4. Resuspend the cytokines following the manufacturers instructions. Calculate the volume of each cytokine to add to the culture as follows:
Culture Volume x cytokine concentration = quantity of cytokine required

EX-VIVO EXPANSION OF CD34 SELECTED PBPC

Quantity of cytokine required) cytokine concentration = volume of cytokine to add
example: Culture volume = 1000 ml, cytokine concentration = 0.3 mg/ml
1000ml x 100ng/ml = 100000ng cytokine or 100 Φg or 0.1 mg
0.1 mg) 0.3 mg/ml = 0.333 ml of cytokine to add

Have another technologist check the calculations and initial the worksheet.

5. Place the 3ml aliquot of media into a 12x75 polypropylene tube labeled Acytokines≅. Remove the volume of each cytokine from the individual vials using a 1cc syringe and 20g needle. Pull additional air into the syringe. Inject the cytokine into the labeled 12x75 tube chasing with air to ensure the entire volume was injected into the tube. After all three cytokines have been added to the tube, draw the contents into a 10 ml syringe using an 18g spinal needle. Pull additional air into the syringe. Remove the needle and attach the syringe to the leur adaptor on the tubing line of the Ex-Vivo Cell Processing bag. Inject the cytokine mixture into the culture bag chasing with air to ensure the entire volume was delivered into the bag.

6. Transfer the CD34 selected cell fraction into a 50 cc conical tube with 8×10^7 cells per tube. Using a 60cc syringe with an 18G needle, pull each 50 ml cell suspension into a syringe. Remove the needle and attach the syringe to the leur adaptor on the tubing line of the Ex-Vivo Cell Processing bag. Inject the cells into the culture bag chasing with air to ensure the entire volume was delivered into the bag.

7. Use the 5ml aliquot of media to rinse the conical tube. Pull the rinse into a 10cc syringe with an 18g spinal needle. Pull additional air into the syringe. Remove the needle and attach the syringe to the leur adaptor on the tubing line of the Ex-Vivo Cell Processing bag. Inject the rinse into the culture bag chasing with air to ensure the entire volume was delivered into the bag.

8. Remove the syringe on the leur adaptor and replace with an interlink injection site.

9. Place the culture bag in a 37°C humidified incubator with 5% CO_2. Record the date and time on the worksheet. Determine the date the culture will be completed.

Culture Maintenance:

10. Check the culture daily. Perform a visual check and record the incubator temperature and %CO_2 on the Ex-Vivo Culture Maintenance Worksheet. If there is any unusal turbidity or media indicator color change, notify the Laboratory Medical Director immediately.

Culture Completion:

11. After 10 days, remove a 0.5 ml aliquot of the cultured cells. Perform a viability and

EX-VIVO EXPANSION OF CD34 SELECTED PBPC

hemocytometer cell count and record on the Ex-Vivo Expansion Culture Completion worksheet. Send the rest of the aliquot to the Clinical Laboratory for a stat gram stain.

12. Install a single processing set on the COBE 2991. Place the purple line behind the SOV solenoid valve. Place all other lines in front of their solenoid valves and clamp with hemostats close to the manifold. Hang a 3L bag of D-PBS on the green line. Make the following instrument settings: Centrifuge speed 3000 rpm
    Superout rate450 ml/min
    Min Agitate Time70 *** Manual Processing Mode!!!
    Superout Volume400 ml
    Valve SelectorV2

13. Label three 600ml transfer packs Supernatant 1, Supernatant 2, and Supernatant 3. Attach two double coupler adaptors to the blue line. Attach the labeled bags to the three spikes. Close all slide clamps except to the bag labeled Supernatant 1.

14. Hang the Ex-Vivo Cell Processing bag on the instrument and spike with the red line.

15. Press Blood In. Remove the hemostat from the red line. When ~ 200 mL have entered the processing bag, press Air Out. Allow the bowl to spin and force the air up the red line. Press Blood In and wait until the processing bag is full. Replace the hemostat on the red line. Press Start/Spin. Set a timer for 4 minutes.

16. When the timer sounds, press Super Out. After 50ml have been expressed, switch the SOV to Collect. Collect about 300 ml of supernatant into the bag then switch the SOV back to SOV. Watch carefully as the rest of the supernatant is expressed. If cells appear to be exiting, immediately press Agitate/Wash In, otherwise, continue to super out until no additional fluid is entering the waste bag, then press Agitate/Wash In.

17. Remove the hemostat from the red line. Allow the processing bag to fill with unprocessed cells. When all the cells have entered the bow, place a hemostat on the clear line. Remove the hemostat from the green line and allow 100-200 mL of PBS to enter the culture bag. Replace the hemostat on the green line. Rinse the culture bag well and remove the hemostat from the clear line. Allow the rinse to enter the processing bowl. Replace the hemostat on the red line. Remove the hemostat from the green line and top off the bowl with PBS. Replace the hemostat on the red line. Press Start/Spin. Set a timer for 4 minutes.

18. When the timer sounds, press Super Out. After 50ml have been expressed, switch the SOV to Collect. Collect about 300 ml of supernatant into the bag labeled Supernatant 2, then switch the SOV back to SOV. Watch carefully as the rest of the supernatant is expressed. If cells appear to be exiting, immediately press Agitate/Wash In, otherwise, continue to super out until no additional fluid is entering the waste bag, then press Agitate/Wash In.

19. Remove the hemostat from the green line and top off the bowl with PBS. Replace the

hemostat on the red line. Press Start/Spin. Set a timer for 4 minutes.

20. When the timer sounds, press Super Out. After 50ml have been expressed, switch the SOV to Collect. Collect about 300 ml of supernatant into the bag labeled Supernatant 3, then switch the SOV back to SOV. Watch carefully as the rest of the supernatant is expressed. If cells appear to be exiting, immediately press Stop/Reset. Otherwise, continue to super out until no additional fluid is entering the waste bag, then press Stop/Reset.

21. Seal all lines on the processing set. Seal the processing bag below the rotating seal and immediately label with the patients name and hospital number. In the laminar flow hood, place a sampling site coupler into the port of the processing bag. Label a 60cc syringe with the patients name and hospital number. Pull 60cc of air into the syringe and transfer into the processing bag. Resuspend the cells and pull into the syringe (measuring the exact volume). Pull 10cc of D-PBS into a 10cc syringe and transfer into the processing bag. Rinse well and pull the rinse into the 60cc syringe. An additional labeled syringe may be needed. Record the volume on the worksheet.

22. Mix well and remove a 0.5ml sample. Perform a cell count and viability. Calculate the total cells available.

23. Calculate the number of cells that would be required to perform the following assays: %CD34, Colony Forming Unit Assay, and 2 cytospins for WBC differential and CD41 by IHC. Multiply the total cells available by 10%. If the number of cells required to perform the assays is greater than 10% of the cells available, perform only the %CD34 assay.

24. Remove 22 ml of Supernatant 1. Innoculate a pair of Bactec vials for sterility culture and send to the Clincial Laboratory. Also send a sample for mycoplasma detection. Label five 4ml cryo vials with the PBPC identification number, date, and supernatant 1. Aliquot 3.6ml into each vial and store at -20°C.

25. Remove 22 ml of Supernatant 2. Label five 4ml cryo vials with the PBPC identification number, date, and supernatant 2. Aliquot 3.6ml into each vial and store at -20°C.

26. Remove 22 ml of Supernatant 3. Label five 4ml cryo vials with the PBPC identification number, date, and supernatant 3. Aliquot 3.6ml into each vial and store at -20°C.

Release Criteria:

27. The product will only be released for infusion if the visual inspection is OK, the stat gram stain is negative, and the viability is greater than 70%.

Preparation for Infusion:

28. Prepare a base label with the component name, component identification number, facility

EX-VIVO EXPANSION OF CD34 SELECTED PBPC

identification, do not irradiate, and store at 20-24°C. Place the completed base label on the syringe containing the cells. Place a label containing the intended recipient=s name and hospital number on the syringe. Have another technologist witness the labeling and sign the worksheet.

29. Prepare an Allogeneic Stem Cell Infusion Requisition with the recipient=s and product information. The product type is PBPC, Ex-Vivo Expanded.

30. Notify the Bone Marrow Transplant Unit Staff that the cells are ready. When the syringe is passed through, the individual taking the syringe (must be the patients nurse or physician) must verbally and visually identify the recipient name, hospital identification and product name with the technologist and document its receipt by signing the Allogeneic Stem Cell Infusion Requisition. Remove the top (white) copy of the requisition and place it in the patient file. Give instructions to place the yellow copy in the patient=s chart and return the bottom (manilla) copy to our laboratory after the infusion is complete.

QUALITY CONTROL

The culture is prepared using sterile technique in a laminar air flow hood.
The culture is checked daily during the incubation.
Culture and sensitivity assays are performed before and after the incubation.
Patient name and history number are confirmed before thawing and dispensing.

REFERENCES

See Appendix B: References in the Stem Cell Engineering Clinical Laboratory Procedure Manual.

Adopted 1/10/97

Approved _____
Elizabeth J. Shpall, M.D.
Director, Stem Cell Engineering Clinical Laboratory

RECIPIENT NAME:_____ DATE:_____

HOSP. NUMBER:_____ WEIGHT:_____kg DIAGNOSIS:_____

LOT NUMBERS & EXPIRATION DATES:

Ex-Vivo Cell Processing bag	_____	interlink injection site	_____
Stem Cell Factor	_____	Defined Medium	_____
Granulocyte-CSF	_____	Sampling site	_____
MGDF	_____	_____	_____

CD34 SELECTED CELL FRACTION DATA:

Product ID number: Date Selected: _____ Tech: _____

Post Selection: Cell Count _____x10^6 c/ml

Volume _____ ml

Total cells available _____x10^6

10% of available _____x10^6

Assay Calculations: check if done

- CFU-GM (1e^4/ml x 5ml) _____ µl 9
- %CD34/3 (flow - 5e^5/tube) _____ µl 9
- 2 cytospins (1x10^5/slide) _____ µl 9

Cells required for assays _____x10^6

CULTURE PREPARATION:

Volume of culture: _____ Number of culture bags: _____

Resuspend cytokines: Date / Time: _____ Tech: _____

Cytokine calculations (per culture bag): Culture Bag Volume = _____ ml

SCF: 100ng/ml x _____ ml = _____ ng = _____ mg of cytokine) _____ mg/ml = _____ ml SCF

(Culture Volume) (SCF stock concentration)

G-CSF: 100ng/ml x _____ ml = _____ ng = _____ mg of cytokine) _____ mg/ml = _____ ml G-CSF

(Culture Volume) (G-CSF stock concentration)

MGDF: 100ng/ml x _____ ml = _____ ng = _____ mg of cytokine) _____ mg/ml = _____ ml MGDF

(Culture Volume) (MGDF stock concentration)

Calculations performed by: _____ Checked by: _____

Check when performed:

9 Add cytokines to Ex-Vivo Cell Processing bag Tech: _____

9 Add cells to Ex-Vivo Cell Processing bag Tech: _____

9 Add rinse to Ex-Vivo Cell Processing bag Tech: _____

9 Place interlink injection site on Ex-Vivo Cell Processing bag Tech: _____

9 Place Ex-Vivo Cell Processing bag in incubator Tech: _____

Time: _____ Date of Culture Completion: _____

RECIPIENT NAME:_____ DATE:_____

STEM CELL ENGINEERING CLINICAL LABORATORY WORKSHEET

Bone Marrow Transplant Program, University of Colorado Health Sciences Center

EX-VIVO EXPANSION WORKSHEET - CULTURE MAINTENANCE

HOSP. NUMBER:_____ WEIGHT:_____kg DIAGNOSIS:_____

UNIT ID NUMBER:_____ NUMBER OF CULTURE BAGS: _____

Day of Culture	Date	Time	Visual Check	Incubator Temperature	Incubator $\%CO_2$	Tech
Day 0 culture initiation						
Day 1						
Day 2						
Day 3						
Day 4						
Day 5						
Day 6						
Day 7						
Day 8						
Day 9						
Day 10 culture completion						

RECIPIENT NAME:_____ DATE:_____

STEM CELL ENGINEERING CLINICAL LABORATORY WORKSHEET

Bone Marrow Transplant Program, University of Colorado Health Sciences Center

EX-VIVO EXPANSION WORKSHEET - CULTURE COMPLETION

HOSP. NUMBER:_____ WEIGHT:_____kg DIAGNOSIS:_____

UNIT IDENTIFICATION: _____

LOT NUMBERS & EXPIRATION DATES:

Transfer set _____ COBE set _____ HSA _____

300ml bag _____ 600ml bag _____ Double coupler _____

1000ml bag _____ D-PBS _____ Sampling site _____

3 way stopcock _____

PRE CULTURE DATA:

Cell Count _____$x10^6$

Volume _____ ml

Total cells _____$x10^9$

%CD34 _____ %

Total CD34 _____$x10^6$ Tech: _____

RELEASE CRITERIA:

		Acceptable Result
Visual Inspection:	_____	OK
Stat Gram Stain Results:	_____	Negative
Viability:	_____	> 70% Tech: _____

POST CULTURE PROCESSING:

PRE: Number of culture bags: _____

[ ] Remove 0.5ml aliquot: viability _____ % Tech: _____

stat gram stain: results:_____ Telephoned by: _____

POST:[ ] Remove 0.5ml aliquot: Cell Count _____$x10^6$

Volume _____ ml

Total cells available _____$x10^9$

10% of available _____$x10^6$

%CD34 _____ %

Total CD34 _____$x10^6$

STEM CELL ENGINEERING CLINICAL LABORATORY WORKSHEET

Bone Marrow Transplant Program, University of Colorado Health Sciences Center

EX-VIVO EXPANSION WORKSHEET - CULTURE COMPLETION

Viability _____ %

Assay calculations: **Check assays performed:**

- CFU-GM ($1e^4$/ml x 5ml)_____ µl 9
- %CD34 (flow - $5e^5$/tube) _____ µl 9
- 2 cytospins ($1e^5$/slide) _____ µl 9

Total cells required for assays _____$x10^6$

REINFUSION DATA: **(Attach laboratory copies of Stem Cell Infusion Requisition)**

Labeled by: _____ **Witness:** _____

Patient identification: **Date / Time dispensed:** _____ **Tech:** _____

APPENDIX B:

Systemic Infusion-Related Toxicity Evaluation

SYSTEM INFUSION-RELATED TOXICITY

PARAMETER	GRADE 0	GRADE 1 (Mild)	GRADE 2 (Moderate)	GRADE 3 (Severe)	Grade 4 (Life-Threatening)
Gastrointestinal Vomiting in 4-hr period	None	1 episode in 4-hr period	2 episodes requiring antiemetics in 4-hr period	3-4 episodes, or requires parenteral support in 4-hr period	≥ 5 episodes or grossly bloody in 4-hr period
Vomiting (in 24 hours)	None	1 episode in 24 hours	2-5 episodes, requires antiemetics in 24 hrs	6-10 episodes, or requires parenteral support in 24 hrs	∃ 10 episodes in 24 hrs or grossly bloody
Diarrhea	None	2-4 stools/day over pretreatment	5-6 stools/day, moderate abdominal cramping	7-9 stools/day, severe cramping or incontinence or requires parenteral support	∃10 stools/day or grossly bloody diarrhea
Renal/Bladder Hemoglobinuria (by dipstick)	None	1+ - 2+	3+ - 4+	Red urine	------

PARAMETER	GRADE 0	GRADE 1 (Mild)	GRADE 2 (Moderate)	GRADE 3 (Severe)	Grade 4 (Life-Threatening)
Creatinine	Within Normal Limits	1.25 - 1.5x normal range	1.5 - 2x normal range	2 - 4x normal range	>4x normal range or requiring dialysis
Liver Bilirubin	Within Normal Limits	1.5 - 2.0x normal range	2.1 - 4.9x normal range	5x - 20x normal range	> 20x normal range
Transaminases	Within Normal Limits	1.5 - 2.0x normal range	2.1 - 4.9x normal range	5x - 20x normal range	> 20x normal range
Alkaline phosphatase	Within Normal Limits	1.5 - 2.0x normal range	2.1 - 4.9x normal range	5x - 20x normal range	> 20x normal range
Metabolic Sodium	Within Normal Limits	2-4 mEq change above or below normal range	4.1 - 8 mEq change above or below normal range	8.1 - 16 mEq change above or below normal range or requiring Rx	>16 mEq above or below normal range or symptoms
Potassium	Within Normal Limits	0.1 - 0.4 mEq change above or below normal range	0.5 - 0.9 mEq change above or below normal range	1.0 - 1.4 mEq change, above or below normal range or EKG changes	> 1.5 mEq change above or below normal range or symptomatic cardiac abnormalities
Hematology		Mild, not requiring	Gross bleeding requiring	Gross bleeding requiring	

PARAMETER	GRADE 0	GRADE 1 (Mild)	GRADE 2 (Moderate)	GRADE 3 (Severe)	Grade 4 (Life-Threatening)
Bleeding	None	transfusion	1-2 units RBCS or additional platelets	3-4 units RBCS	Requires >4 units RBCS
Infection	None	No active treatment	Localized infection, requires antibiotic	Systemic infection, requires IV antibiotic or antifungal therapy	Sepsis. Specify site
Immunological Allergy	None	Transient rash, drug fever <38c (100.4F)	Urilicaria drug fever ∃38 (100.4F) mild bronchospasm	Serum sickness, bronchospasm, requires parenteral meds	Anaphylaxis
Respiratory	None	Dyspnea	Persistent cough and/or wheezing and/or lRR 2x baseline and/or mild hypoxemia (O2 sat 9 2-4% points from baseline)	Stridor and/or hypoxemia requiring supplemental O2. O2 sat 9 ∃5% points from baseline	Requires ventilatory support and/or positive VQ scan/pulmonary arteriogram
Cardiac Rate and Rhythm	None	HR > 10 bpm (below	Other arrhythmias not	Bradycardia and/or	Life-threatening

PARAMETER	GRADE 0	GRADE 1 (Mild)	GRADE 2 (Moderate)	GRADE 3 (Severe)	Grade 4 (Life-Threatening)
		baseline) or bradycardia (<60 bpm)	requiring Rx	arrhythmias requiring medical intervention, including change in rate of marrow infusion	arrhythmias requiring aggressive intervention
Hypertension	None or no change	Asymptomatic transient increase by > 20 mm Hg (D) or to > 150/100 if previously WNL. No treatment required	Recurrent or persistent increase by > 20mm Hg (d) or to > 150/100 if previously WNL. No treatment required.	Requires therapy	Hypertensive crisis
Hypotension	None or no change	Changes requiring no therapy (including transient orthostatic hypotension)	Requires fluid replacement	Requires fluid replacement and vasopressors and/or change in rate of marrow infusion	Shock
Febrile Reaction Temperature	None	37.1 - 38.0 C (98.7 - 100.4 F)	38.1 - 40.0 C (100.5 - 104.0 F)	>40.0 C > 24 hrs (>104.0 F < 24 hrs)	Septic Shock

PARAMETER	GRADE 0	GRADE 1 (Mild)	GRADE 2 (Moderate)	GRADE 3 (Severe)	Grade 4 (Life-Threatening)
Chills	None	Mild	Shaking	Shaking requiring Rx	------
Sweats	None	Mild	Drenching	------	------
Skin	None or no change	Transient rash or mucosal congestion	Urticaria, generalized rash	Requires Rx	Exfoliative dermatitis

Consent Form Approval

Date: _____ Valid Through: _____
Allan Prochazka, MD/Steve Bartlett, R.Ph. Co-Chairs, COMIRB
Christopher Kuni, MD/Ken Easterday, RPh.. Co-Chairs, COMIRB
Adam Rosenberg, MD/David Lawellin, Ph.D. Co-Chairs, COMIRB

COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD
SUBJECT CONSENT FORM

TITLE: **Transplantation of Breast Cancer Patients with Growth Factor-Mobilized Peripheral Blood Progenitors which are CD34-Selected and Expanded Ex Vivo Prior to Infusion: COHORTS 1 AND 2 (IRB #98-040)**

Principal Investigator: Elizabeth J. Shpall, M.D.
July 9, 2001

You are being asked to participate in a research study involving an investigational technique where a portion of your peripheral blood progenitor cell transplant will be treated in the laboratory before it is given back to you. We anticipate enrolling 100 patients on this research study.

The first goal of this study is to determine whether we can reduce the amount of breast cancer in your peripheral blood progenitor cells (PBPCs).

The second goal of this study is to determine whether we can speed up your marrow recovery after high-dose therapy, by increasing the number of blood-forming cells in the laboratory before you receive them.

Your doctors have already discussed with you the procedures for high-dose chemotherapy administration and PBPC transplantation for your breast cancer. You have signed a separate consent form agreeing to those procedures.

STUDY RATIONALE

Peripheral blood is a mixture of different types of cells, of which only a small fraction called the ACD34-positive (+) stem cells≅ are necessary for successful transplantation. The remaining unwanted cells include red cells, platelets, and breast cancer cells. There are several devices which isolate the CD34+ stem cells needed for transplant and attempt to leave the unwanted cells including breast cancer cells behind. CD34+ stem cells isolated with such a device, built by CellPro Inc. (Bothell, Washington), have successfully produced marrow recovery in more than 200 patients treated in the University of Colorado Bone Marrow Transplant Program. However, in many of these patients there were still breast cancer cells left in the CD34+ stem cell fraction. Laboratory experiments have shown that if such CD34+ stem cell fractions contaminated with breast cancer are grown in the laboratory for 10 days, the vast majority of breast cancer cells do not survive, while the number of normal stem cells increases substantially.

The purpose of this study is to:

1. Determine whether growing the CD34+ stem cells in the laboratory for 10 days prior to transplant can remove all detectable breast cancer cells
2. Determine whether growing the CD34+ stem cells in the laboratory for 10 days prior to transplant will provide more rapid bone marrow recovery than the standard stem cells which are not grown in the laboratory prior to infusion.

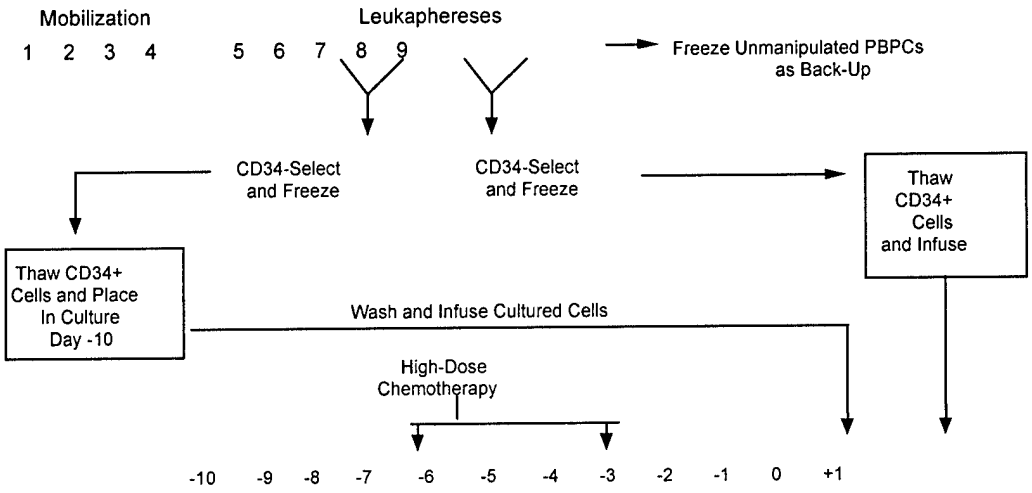
In order to test this, your CD34+ stem cells will be isolated from your peripheral blood fractions using a newer CD34-selection device built by Nexell Inc. CD34+ peripheral blood stem cells isolated with this device have successfully transplanted >400 patients in the U.S. and Europe. The device has been used at the University of Colorado to isolate cord blood stem cells that successfully engrafted patients, but has not been used previously for breast cancer patient studies. The CD34+ cells will be grown in the laboratory for 10 days, and then given back to you after the chemotherapy is administered. We will count the number of breast cancer cells detected before and after the 10 day growth period. We will also measure whether these Amanipulated≅ stem cells grow and produce new blood cells inside your body in a timely fashion

STUDY DESIGN

If you choose to participate, this study will enroll up to 100 patients into each of two different groups. The group to which you are assigned will depend upon when you are ready for your transplant. Previous patients received only CD34+ PBPCs isolated with the Nexell device and adequate marrow recovery was demonstrated. You will be assigned to Group 1 or 2. Group 1 patients will receive CD34+ PBPCs, a fraction of which have been grown in the laboratory. If adequate marrow recovery is demonstrated in the Group 1 patients, the study will progress to Group 2 where the patients will receive only the PBPCs which are grown in the laboratory prior to transplant.

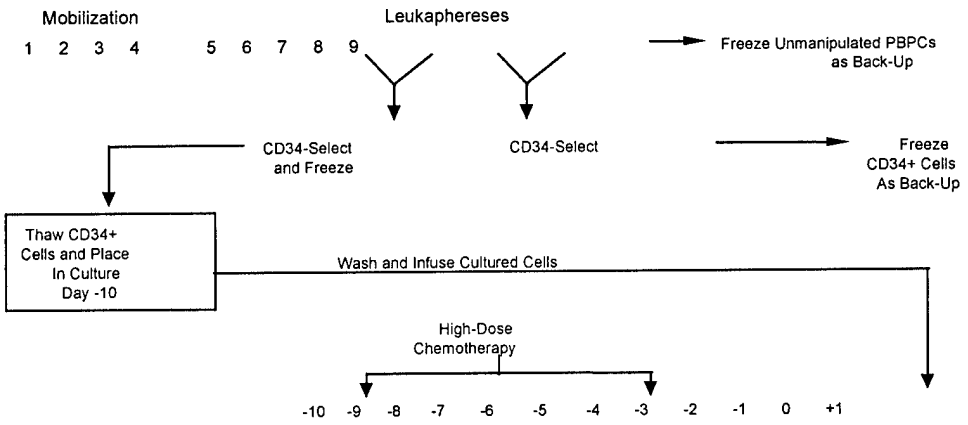
Group 1: CD34+PBPCs Before and After Growth in the Laboratory. You will receive growth factor(s) daily and undergo leukaphereses on consecutive days 5, 6, 7, 8, and 9. The day 5 and 6 PBPCS will be combined, CD34-selected and cryopreserved on day 6. The day 7 and 8 PBPCS will be combined, CD34-selected and cryopreserved on day 8.The combined CD34+ PBPC product (5/6 or 7/8) with the highest number of CD34+ PBPCS will be grown in the laboratory. On chemotherapy day -10, the designated combined CD34+ PBPC product will be thawed and placed in the laboratory to grow. On days -6 through -3, high-dose therapy will be administered. On day 0, you will receive the PBPCs which will have been growing in the laboratory for 10 days. On day +1 you will receive the other frozen CD34+ PBPC product which will be thawed and immediately infused. You will receive G-CSF daily from day 0 until you achieve an ANC of 5,000.

Ex Vivo Expansion of Growth Factor Mobilized PBPCs
Cohort 1



Group 2: CD34+PBPCs Grown in the Laboratory. You will receive growth factor(s) daily and undergo leukaphereses on consecutive days 5, 6, 7, 8, and 9. The day 5 and 6 PBPCs will be combined, CD34-selected and cryopreserved on day 6. The day 7 and 8 PBPCs will be combined, CD34-selected and cryopreserved on day 8.The combined CD34+ PBPC product (5/6 or 7/8) with the highest number of CD34+ PBPCS will be grown in the laboratory. On chemotherapy day -10, the designated combined CD34+ PBPC product will be thawed and placed in the laboratory to grow. On days -6 through -3, high-dose therapy will be administered. On day 0, you will receive the PBPCs which will have been growing in the laboratory for 10 days. You will receive G-CSF daily from day 0 until you achieve an ANC of 5,000.

Ex Vivo Expansion of Growth Factor Mobilized PBPCs
Cohort 2



_____ Pt. Initials

Back-Up Peripheral Blood Progenitor Cell Fraction. While many patients who received CD34+ stem cells isolated with the CellPro device had acceptable marrow recovery, there is a risk that CD34+ stem cells isolated with the Nexell device and then grown in the laboratory will fail to function. For this reason, once peripheral blood progenitor cells sufficient for the transplant have been collected, you will have additional PBPCs collected which will be cryopreserved as an unmanipulated back-up fraction should the cells isolated with the Nexell device and grown in the laboratory fail to function.

RISKS OF TREATING THE PBPCs IN THE LABORATORY PRIOR TO TRANSPLANTATION

The cells which have been growing for 10 days could produce side effects when infused including fever, chills, and shortness of breath. In approximately 30 patients who have received marrow or peripheral blood stem cells grown in laboratories in the United States or Europe prior to infusion, fever was the only side effect reported. There were no side effects seen in five patients who received cord blood grown in the laboratory at the University of Colorado prior to transplant.

There is a risk that the blood cells could become infected during the 10-day treatment. Your doctors will check the cells for infection 24 hours before they are given back to you. If they are infected with certain (dangerous) organisms, the treated cells will **not** be given back to you. Although very unlikely, there is a small chance (less than 0.1%) that even though the tests show no infection, one might be present and upon infusion could produce a serious or even fatal infection in your blood.

There is a risk that this procedure will slow the blood cell recovery which would normally occur. Delayed marrow recovery increases the risk of bleeding (due to inadequate platelets), infection (due to inadequate white blood cells), and possibly death. In addition, you may require more platelet and packed red cell transfusions than patients who do not have their blood cells treated. It is also possible that both the treated and/or the untreated blood cells will not be satisfactory, and marrow recovery will not occur. If this happens, it may be necessary to infuse your back-up PBPC fraction. Although the availability of the back-up fraction reduces the risk that your marrow won't recover, it does not eliminate it.

You must realize that significant risks, including the possibility of death, accompanies this treatment program. The risks and benefits for you as an individual should be carefully weighed before agreeing to participate in this program. There may also be risks that are currently unforseeable.

TREATMENT ALTERNATIVES

You could receive a transplant at the University of Colorado with PBPCs that are not treated prior to infusion.

BENEFIT OF THE STUDY

The study may be of no benefit to you.

COSTS OF THIS PROGRAM

The materials used in the peripheral blood stem cell growth procedure will be provided by Amgen, Inc. free of charge. Other costs of the stem cell processing and transplant program must be borne by you, your insurance carrier, or your estate, as they are applicable, whether you participate in this study or not. The Bone Marrow Transplant Program personnel will aid you in determining the extent of your insurance coverage for this program.

OBTAINING ADDITIONAL INFORMATION

You are encouraged to ask questions at any time if any part of this program is unclear to you, and you have the right to have your questions answered. Any significant new findings which might develop after you consent will be discussed with you. Drs. Elizabeth Shpall, Scott Bearman, Roy Jones Peter McSweeney, and Yago Nieto, are available to answer your questions at (303) 372-9000.

STUDY WITHDRAWAL

If you decide to participate in this study, you are free to withdraw from the entire treatment program at any time prior to high-dose chemotherapy administration without compromising further care from your physicians.

If your physicians feel at any time that continued participation in this program is not in your best interest, they may decline to administer the remaining components of this program without your consent.

CONFIDENTIALITY

When the results of this or associated studies are reported in medical meetings or the medical literature, the identity of all participants is withheld. Government agencies, such as the Food and Drug Administration, or Amgen, Inc., who is sponsoring this program, or other bone marrow transplant centers participating in this trial have the right to inspect medical records which contain your name. Confidentiality of your medical records is maintained according to Hospital policy. A report of the results of this study may be published or sent to the appropriate health authorities in any country in which the drug may ultimately be tested, but your name will not be disclosed in these documents. Study records will be maintained indefinitely for the purpose of analysis and follow-up.

COMPENSATION FOR INJURIES SUSTAINED DURING THIS PROGRAM

Immediate necessary care is available if you are injured because of participation in this program. However, there is no provision for free medical care or for monetary compensation for such injury.

For further information concerning this or your rights as a subject participating in this research project, please call the COMIRB, (303)724-1055.

AUTHORIZATION

I have read this paper about the study or it was read to me. If necessary, the description of the study was translated and explained to me in a language that I understand better than English. I know what will happen, both the possible good and bad (benefits and risks). I understand the discomforts, inconveniences, and risks of this study. I have been given the opportunity to ask questions and have these questions answered prior to the signing of this consent form. I choose to be in this study. I know I can stop being in the study and I will still get the usual medical care. I will receive a copy of this consent form.

WITNESSING AND SIGNATURE

Patient's Name (Please Print):

_____ _____
Medical Record #

I agree to participate in this study.

Patient's Signature:

_____ _____
Date

Physician's Signature:

_____ _____
Date

Witness Signature:

_____ _____
Date

_____ Pt. Initials

EXPERIMENTAL SUBJECT'S BILL OF RIGHTS

Any person who is requested to consent to participate as a subject in a research study involving a medical experiment or who is requested to consent on behalf of another has the right to:

1. Be informed of the nature and purpose of the experiment.

2. Be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized.

3. Be given a description of any attendant discomforts and risks reasonably to be expected from the experiment.

4. Be given an explanation of any benefits to the subject reasonably to be expected from the experiment, if applicable.

5. Be given a disclosure of any appropriate alternative procedures, drugs or devices that might be advantageous to the subject, and their relative risks and benefits.

6. Be informed of the avenues of medical treatment, if any, available to the subject, and their relative risks and benefits.

7. Be given an opportunity to ask any questions concerning the experiment or the procedure involved.

8. Be instructed that consent to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation in the medical experiment without prejudice.

9. Be given a copy of any signed and dated written consent form used in relation to the experiment.

10. Be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

JOINT DIVISION OF ENDOCRINOLOGY, METABOLISM & DIABETES AND
PROGRAM IN HORMONAL CANCERS RESEARCH CONFERENCE

2000-2001 Schedule
Room 623 Biomedical Research Building
Tuesdays, 4:00 p.m.
Unless otherwise noted**

DATE	SPEAKER	TITLE
September 5	**Robert Lustig, M.D.** Associate Professor Department of Pediatrics Le Bonheur Children's Medical Center	**"Primary Insulin Hypersecretion: A specific and Remediable Form of Obesity"**
September 12	**Gary Johnson, Ph.D.** Professor Department of Pharmacology UCHSC	**"Signal Transduction Pathways Controlling Chemotherapy-Induced Apoptosis: Genotoxin-Induced Paracrine Secretion of TRAIL"**
September 19	**Scott Summers, Ph.D.** Assistant Professor Colorado State University	**"Regulation of Insulin Action by Ceramide"**
September 26	**Karen Newell, Ph.D.** Assistant Professor UCCS	**TBA**
October 3	**Twila A. Jackson, Ph.D.** Fellow Obstetrics and Gynecology UCHSC	**"FGF Signal Transduction in the Pituitary"**
October 10	**Mark L. Dell'Acqua, Ph.D.** Assistant Professor Pharmacology UCHSC	**"Regulation of the AKAP79 Signaling Scaffold"**
October 17	**Steven Maier, Ph.D.** University of Colorado – Boulder	**"Cytokines and the Regulation of the HPA Axis"**
October 24	**A. Pratap Kumar** Associate Scientist AMC Cancer Research Center	**"Novel Role for Estrogenic Metabolites in Prostate Cancer Chemoprevention"**

DATE	SPEAKER	TITLE
October 31	**Kimberly Leslie, M.D.** Associate Professor Obstetrics and Gynecology UCHSC	**"Progesterone-Induced Pathways to Differentiation in the Endometrium"**
November 7	**TBA**	
November 14	**Margaret Shupnik, Ph.D.** University of Virginia Health Sciences Center Charlottesville, VA	**"Dynamic Estrogen Regulation of Pituitary Function by a Novel Estrogen Receptor"**
November 21	**HOLIDAY** – No Seminar	
November 28	**Lori Lee Larson, M.D.** Senior Fellow Division of Endocrinology, Metabolism & Diabetes UCHSC	**"Retinoids and Thyroid Cancer"**
December 5	**Jeffrey L. Bennett, M.D., Ph.D.** Assistant Professor Neurology and Ophthalmology UCHSC	**"Neurotrophin Function in the Mammalian Eye"**

PLEASE NOTE: An updated schedule will be sent out at a later date.

University of Colorado Cancer Center

Program in Hormone-Related Malignancies

Spring 2001 Seminars

Room 623 Biomedical Research Building

Tuesdays, 4:00 p.m.

Unless otherwise noted **

DATE	SPEAKER	TITLE
January 16	**Lori Lee Larson, M.D.** Senior Fellow Division of Endocrinology, Metabolism & Diabetes UCHSC	**"Retinoids & Thyroid Cancer"**
January 23	**Christina Finlayson, M.D.** Assistant Professor Surgery **Meenakshi Singh, M.D.** Assistant Professor Pathology	**"Background of Neoadjuvant Chemotherapy for Breast Cancer"** **"Mib-1 and VEGF Expression in Pre- and Post-Chemotherapy Breast Cancer"**
January 30	**Ian McNiece, Ph.D.** Director of Research Bone Marrow Transplant Program UCH	**"The Role of Dendritic Cells in Immune Reconstitution in Breast Cancer"**
February 13** **2:00 p.m.**	**Matthew Ringel, M.D.** Washington Hospital Center Washington DC	**"The Role of AKT Signaling in Thyroid Neoplasia"**
February 20	**Peter Jones, Ph.D.** Assistant Professor Pediatrics UCH	**"Control of Cell Growth and Differentiation by the Extracellular Matrix: Studies with the Oncofetal Protein Tenasin-C"**
February 27	**Saraswati Sukamar, Ph.D.** Director of Basic Research John's Hopkins Oncology Center Baltimore, MD	**"HOXA5-A Key Role in Mammary Development and Neoplasia?"**
March 6** **2:00 p.m.**	**Bruce Lessey, M.D., Ph.D.** Professor Dept. Obstetrics and Gynecology University of North Carolina	**"Endocrine and Paracrine Regulators of Uterine Receptivity: A Tale of Two Proteins"**

DATE	SPEAKER	TITLE
March 20	**David Bain, Ph.D.** Assistant Professor Division of Endocrinology, Metabolism & Diabetes UCHSC	**"Structural Studies of Human Progesterone Receptor"**
April 10	**Philip Zeitler, M.D., Ph.D.** Assistant Professor Pediatrics UCHSC	**"Growth Hormone Releasing Hormone and Breast Cancer"**
May 1	**Arthur Gutierrez-Hartmann, M.D.** Professor of Medicine Division of Endocrinology, Metabolism & Diabetes UCHSC	**"The Role of ESX in Breast Cancer"**
May 8	**John Pawlowski, Ph.D.** Instructor in Medicine Division of Endocrinology, Metabolism & Diabetes V.A. Medical Center	**"Mechanisms of Transcriptional Repression by Liganded Steroid Receptors"**
May 22	**Jason D. Prescott** Breast Cancer Training Program	**"Elucidating Function: In Vivo Analysis of the Ets Transcription Factor ESX"**
	Aaron C. Spalding Breast Cancer Training Program	**"Genotoxins Induce Paracrine Secretion of TRAIL Committing Epithelial Cells to Apoptosis"**
May 29	**Christine C. Wu** Breast Cancer Training Program	**"Direct Proteomic Analysis of Breast Tumors using Multi-Dimensional Chromatography coupled to Tandem Mass Spectrometry"**
	Steven Rosinski Breast Cancer Training Program	**"T-cell Recovery Following Stem Cell Transplantation"**

Andrew P. Bradford, Ph.D.
Seminar Coordinator
Tel: (303) 315 4146
Andy.Bradford@uchsc.edu

DOD PROGRAM IN BREAST CANCER RESEARCH

JOURNAL CLUB – 2000/2001

The Mammary Journal Club occurs every 2nd Friday at 4:00 PM in the Physiology Conference Room (MS 3837). Topics encompass all aspects of the fundamental biology of the mammary gland and breast cancer.

Date	Presenter	Topic/Article
Nov. 10	Margaret Neville, Ph.D.	Fata *et al.*, the osteoclast differentiation factor osteoprotegerin-ligand is essential for mammary gland development. Cell 103, 41-50.
Jan. 12	Jason Prescott (student)	Biswas *et al.*, Epidermal growth factor-induced nuclear factor kB activation: A major pathway of cell cycle progression in estrogen-receptor negative breast cancer cells.
Mar. 9	Brigitte Troskie, Ph.D.	
Apr. 13	Rick Metz	The steroid receptor coactivator SRC-3 is required for normal growth, puberty, female reproductive function and mammary gland development. PNAS 97(12):6379-6384.
May 11	Heide Ford, Ph.D.	Shekhar *et al.*, Breast stroma plays a dominant regulatory role in breast epithelial growth and differentiation: Implications for tumor development and progression. Cancer Research Advances in Brief, Feb. 2001, p. 1320-1326.